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METHOD FOR DOWN-REGULATING IL-5 ACTIVITY

FIELD OF THE INVENTION

The present invention relates to improvements in therapy and prevention of conditions characterized by an elevated level of eosinophil leukocytes, *i.e.* conditions such as asthma and other chronic allergic diseases. More specifically, the present invention provides a method for down-regulating interleukin 5 (IL-5) by enabling the production of antibodies against IL-5 thereby reducing the level of activity of eosinophils. The invention also provides for methods of producing modified IL-5 useful in this method as well as for the modified IL-5 as such. Also encompassed by the present invention are nucleic acid fragments encoding modified IL-5 as well as vectors incorporating these nucleic acid fragments and host cells and cell lines transformed therewith. The invention also provides for a method for the identification of IL-5 analogues which are useful in the method of the invention as well as for compositions comprising modified IL-5 or comprising nucleic acids encoding the IL-5 analogues.

BACKGROUND OF THE INVENTION

Asthma is a common disease of the airways, affecting about 10% of the population. The present treatments is primarily based on the administration of steroids and represents a market value exceeding well over a billion dollars. For yet unknown reasons the incidence and morbidity of asthmatics have increased worldwide over the past two decades. Today, an improved understanding of the immunological mechanisms involved in asthmatic conditions combined with an explosive development in biotechnology provides a new basis for the development of alternative and perhaps better strategies for treatment.

A general feature in the pathogenesis of asthma and other chronic allergic diseases has proven to be elevated numbers of eosinophils, especially in the bronchial mucosa of the lungs. Upon activation eosinophils secrete a number of mediators that are actively involved in the inflammatory airway response. In the activation of eosinophils, interleukin 5 (IL-5) plays an important role.

IL-5 is a cytokine found in many mammalian species and among others both the human and the murine gene for IL-5 have been cloned (Tanabe *et al.*, 1987, Campbell *et al.*, 1988). The human gene consists of four exons with three introns positioned at chromosome 5 and codes for a 134 amino acid residue precursor, including a 19 amino acid N-terminal leader sequence. Post translational cleavage generates the mature 115 amino acid residue protein (SEQ ID NO: 1). The murine IL-5 (mIL-5) gene similarly codes for a 133 amino acid residue pre-cursor with a 20 amino acid leader sequence. The processed mature mIL-5 is thus 113 amino acid residues long (SEQ ID NO: 12), missing two N-terminal amino acid residues by alignment with human IL-5. The amino acid sequences of hIL-5 and mIL-5 are 70% identical compared to 77% at nucleotide level of the coding regions (Azuma *et al.*, 1986). Higher similarity was reported within human primates; 99% identity is reported for the coding regions of the human and the Rhesus monkey nucleotide sequences (Villinger *et al.*, 1995).

The human amino acid sequence has two potential N-glycosylation sites and the murine three. Human IL-5 has been shown to be both N-glycosylated as well as O-glycosylated at Thr 3. Studies of hIL-5 has demonstrated that the glycosylation is not necessary for the biological activity even though the stability seems to be affected by de-glycosylation (Tominaga *et al.*, 1990; Kodama *et al.*, 1993).

Structure of IL-5

The active IL-5 is a homo-dimer and the 3-dimensional structure of recombinant hIL-5 has been determined by X-ray crystallography (Milburn *et al.*, 1993). The 2 monomers are organised in an antiparallel manner and covalently bound by two interchain disulfide bridges (44-87' and 87-44'), thus engaging all 4 cysteines of the 2 monomers.

The secondary structure of the monomers consists of 4 α-helices (A-D) intermitted by 3 linking regions (loops) including two short stretches of β-sheets. This 4α helix bundle is known as the "common cytokine fold", which has also been reported for IL-2, IL-4, GM-CSF, and M-CSF. But all these are monomers and the homodimer-structure in which the D-helix completes the 4α helix motif of the opposite monomer is unique to IL-5.

The native monomers alone has been shown to be biologically inactive (for reviews see Callard & Gearing, 1994; Takutsu *et al.*, 1997). It is nevertheless possible to produce a modified recombinant biologically active monomer by inserting 8 additional amino acid residues in loop 3, connecting the helices C and D. This enables helix D to complete the 4 helix structure within one polypeptide chain and thus enable the monomer to interact with its receptor (Dickason & Huston, 1996; Dickason *et al.*, 1996).

The IL-5 receptor is primarily present on eosinophils and it is composed of an α-chain and a β-chain. The α-chain of the receptor is specific for IL-5 and the β-chain, which assure high-affinity binding and signal transduction, is shared with the hetero-dimer receptors for IL-3 and GM-CSF. The sharing of a receptor component could be the reason for the cross-competition seen between IL-5, IL-3 and GM-CSF (for review, see Lopez *et al.*, 1992). However, it was recently demonstrated that the regulation of the IL-5R is distinct from the regulation of the

IL-3R and the GM-CSFR, further indicating a highly specialised role of IL-5 in the regulation of the eosinophilic response (Wang *et al.*, 1998).

The C-terminal part of IL-5 seems to be important in both binding to the IL-5R and for the biological activity, since removal of more than two C-terminal amino acid residues results in a decline in both the binding affinity to the IL-5 R and in the biological activity in an IL-5 bioassay (Proudfoot *et al.*, 1996). Other residues have also been found to be important for binding to the receptor, such as Glu12, which is involved in binding to the β-chain, while the Arg90 and Glu109 residues are involved in the binding to the α-chain of the receptor. In general, binding to the IL-5R seems to occur in regions overlapping helices A and D, where helix D is primarily responsible for the binding to the specific IL-5R α -chain (Graber *et al.*, 1995; Takastsu *et al.*, 1997).

IL-5's homology to other proteins

The two 4-helix domain motifs seen in the homodimer has strikingly similar secondary and tertiary structure as compared to the cytokine fold found in GM-CSF and M-CSF, IL-2, IL-4 and human and porcine growth hormone (Milburn *et al.*, 1993). However, even though striking similarities are also observed in the intron/exon organisation and position of cysteines (Tanabe *et al.*, 1987; Cambell *et al.*, 1988) suggesting a phylogenetic relationship with IL-2, IL-4 and GM-CSF, no significant homology with any of these or other cytokines is observed from the amino acid sequence.

Biological activity of IL-5

IL-5 is mainly secreted by fully differentiated Th2 cells, mast cells and eosinophils (Cousins *et al.*, 1994; Takutsu *et al.*, 1997). It has been shown to act on eosinophils, basophils,

cytotoxic T lymphocytes and on murine B cells (Callard & Gearing, 1994; Takutsu *et al.*, 1997). The effects of IL-5 on human B cells are still a matter of controversy. Augmentation of immunoglobulin synthesis under certain circumstances and binding to a variety of human B cell lines have been demonstrated. Even though mRNA for the hIL-5R has been found in human B-cells, the actual presence of the receptor on these cells has still to be verified (Baumann & Paul, 1997; Huston *et al.*, 1996).

The actions of IL-5 on eosinophils include chemotaxis, enhanced adhesion to endothelial cells, activation and terminal differentiation of the cells. Furthermore it has been demonstrated that IL-5 prevents mature eosinophils from apoptosis (Yamaguchi *et al.*, 1991). These findings have contributed to the present concept of IL-5 as being the most important cytokine for eosinophil differentiation (Corrigan & Kay, 1996; Karlen *et al.*, 1998).

Physiologically, IL-5 and its associated eosinophil activation is considered to serve a protective role against helminthic infections and possibly against certain tumours, since these diseases are typically accompanied by peripheral blood eosinophilia (Takutsu *et al.*, 1997; Sanderson *et al.*, 1992). It is, however, somewhat speculative as in two studies the authors failed to show any effect beside eosinophil down-regulation following administration of antibodies against IL-5 on the immunity (e.g. IgE levels) against *Nippostrongylus braziliensis* or *Schistosoma mansoni* in mice infected with these parasites (Sher *et al.*, 1990; Coffman *et al.*, 1989).

IL-5 transgenic and "knock-out" animals

Studies of transgenic mice expressing IL-5 or knock-out mice deficient for IL-5 have given further knowledge of the physiological role of IL-5.

Several IL-5 transgenic mice have been reported:

A transgenic mouse expressing the IL-5 gene in T cells was reported to have an increased white blood cell level characterised by expansion of B220+ B lymphocytes and profound eosinophilia. This was accompanied by a massive peritoneal cavity cell exudate dominated by eosinophils and infiltration of eosinophils in nearly all organ systems (Lee *et al.*, 1997a).

Another transgenic mouse, expressing the IL-5 gene under control of a metallothionin promotor was characterised by an increase in the serum levels of IgM and IgA, a massive eosinophilia in peripheral blood and many other organs accompanied by the expansion of a distinctive CD5+ B cell population, which produce auto-antibodies (Tominaga *et al.*, 1991).

A third study involved a transgenic mouse constitutively expressing IL-5 in the lungs. These animals developed pathophysiological changes resembling those of human asthma, including eosinophil invasion of peribronchial spaces, epithelial hypertrophy and increased mucus production. Furthermore, development of airway hyper responsiveness was seen in the absence of antigens (Lee *et al.*, 1997b).

IL-5-deficient mice ('knock-out' mice) have also been studied. These mice (C57BL/6) have no obvious signs of disease and are fertile. The immunoglobulin levels and the specific antibody responses to DNP-OVA were normal. Basal levels of eosinophils are produced, but are 2-3 times lower than in control animals, indicating that eosinophils can be produced in the complete absence of IL-5. When these mice were infected with *Mesocestoides corti* the eosinophilia normally seen was abolished and this absence of eosinophilia did not affect the worm burden produced by this parasite (Kopf *et al.*, 1996).

In a study by Foster et al. (1996), the effect of IL-5 knock-out on a common model of atopic airway inflammation was investigated. Sensitisation and aerosol challenge of mice with ovalbumin normally result in airway eosinophilia, airway hyperreactivity to β-methacholin and extensive lung damage analogous to that seen in asthma. In the IL-5 deficient mice the eosinophilia, airway hyperreactivity and lung damage were abolished. When IL-5 expression in these mice was reconstituted, the aero-allergen induced eosinophilia and airway dysfunction were restored.

Pathophysiologic role of IL-5

Asthma affect about 10% of the population worldwide and for yet unknown reasons the incidence and morbidity have increased over the past two decades (Ortega & Busse, 1997). It is a chronic airway disease characterised by recurrent and usually reversible air flow obstruction, inflammation and hyper responsiveness (Moxam and Costello, 1990). This produces symptoms of wheezing and breathlessness, which in severe cases can be fatal.

The animal experiments referred to above using transgenic mice constitutively expressing IL-5 in the lungs (Lee *et al.*, 1997a) and the IL-5 deficient "knock-out" mice (Foster *et al.*, 1996) strongly implicate a crucial role of IL-5 in the pathogenesis of asthma. Further evidence supporting this can be deduced from several studies including asthmatic individuals.

Eosinophilia has been identified in bronchoalveolar lavage (BAL) fluid and in bronchial mucosal biopsies of subjects with asthma and correlates with disease severity. Several eosinophil products have been identified in the BAL fluid of patients with asthma and numbers of peripheral blood eosinophils correlate with asthma severity (Ortega & Busse 1997).

IL-5 serum concentration was found to be elevated (median concentration 150 pg/ml) in 15 out of 29 patients with chronic severe asthma as compared to control subjects (Alexander *et al.*, 1994).

In another study involving both non-atopic and atopic asthmatics, it was found that an enhanced IL-5 production by helper T cells seems to cause the eosinophilic inflammation of both atopic and non-atopic asthma (Mori *et al.*, 1997).

Other results also indicate that IL-5 has a distinct role in other atopic diseases. Allergen induced systemic episodes in individuals with allergic rhinitis has recently been shown to correlate to allergen induced IL-5 synthesis rather than IgE (Ohashi *et al.*, 1998). The correlation of atopic reactions is also demonstrated in a study by Barata *et al.* (1998) in which a significant expression of IL-5 by T-cells in a cutaneous late phase reaction is demonstrated.

These and other results have led several authors as Corrigan & Kay (1996), Danzig & Cuss (1997) to identify and recommend IL-5 as a primary target in the development of a better treatment for asthma and atopic diseases involving eosinophilic inflammation. Chronic tissue damaging hypereosinophilia induced by parasitic infection, topical pulmonary eosinophilia and hypereosinophilic syndrome are examples of other pathogenic conditions that could be addressed by IL-5 down regulation.

***In vivo* demonstration of the role of IL-5**

In several studies with rodent models of asthma it has been shown that treatment with monoclonal antibodies against IL-5 (anti-IL-5 mAb) results in dose-related inhibition of eosinophilia, as compared to non-treated controls (Nagai *et al.*, 1993a & b; Chand *et al.*, 1992; Coeffier *et al.*, 1994; Kung *et al.*, 1995; Underwood *et al.*, 1996). In the study by Nagai *et al.*

(1993a) the effect was also observed by treating the sensitised Balb/c mice with soluble IL-5 receptor α.

In one study with Balb/c mice (*Hamelmann et al.*, 1997) and four studies with guinea pigs it was additionally shown that anti-IL-5 mAb could inhibit airway hyperreactivity elicited with various substances in antigen sensitised animals (Mauser *et al.*, 1993; Akutsu *et al.*, 1995; van Oosterhout *et al.*, 1995 & 1993). In some of the studies beneficial effects (cf. table 1) of the anti-IL-5 mAb treatment were also observed microscopically (Mauser *et al.*, 1993; Akutsu *et al.*, 1995; Kung *et al.*, 1995). Importantly, in the study by Kung *et al.* (1995) a reduction of pulmonary inflammation in B6D2F1 mice was seen both when anti-IL-5 mAb was administered hours before antigen challenge and also when administered up to five days after antigen challenge, indicating that the effect of anti-IL-5 mAb may be both prophylactic and therapeutic for airway inflammation. This effect, however, was not observed by Underwood *et al.* when guinea pigs were given anti-IL-5 mAb two hours after antigen challenge (Underwood *et al.*, 1996).

In a study using a monkey model of asthma, Mauser et al. (1995) reported an inhibition of airway hyper-reactivity after antigen challenge, when rat anti mouse-IL-5 mAb was given 1 hour before antigen challenge. In addition, there was 75% reduction in the number of eosinophils in bronchoalveolar lavage (BAL) of antibody treated animals, as compared to non-treated controls. The effects on eosinophilia and hyperresponsiveness of anti-IL-5 mAb was seen for up to three months after treatment (Mauser *et al.*, 1995). Regarding allergic hyperresponsiveness, the results from studies by Nagai *et al.* (1993a and 1993b) document no reduction in hyperresponsiveness in conjunction to a reduction of eosinophil numbers in BAL.

All anti-IL-5 mAb *in vivo* experiments mentioned so far have been done with rat-anti-mouse monoclonal antibodies. Egan *et al.*

(1995) have reported experiments using humanised rat-anti-human IL-5 monoclonal antibodies, called Sch 55700. These mAb's, inhibited lung lavage eosinophilia by 75% at a dose of 0,3 mg/kg when administered to sensitised monkeys. When Sch 55700 was given at 1 mg/kg in allergic mice, inhibition of airway eosinophilia was also observed.

Treatment of asthma at present and in the future

The current treatment of asthma is, as mentioned, corticosteroids which, by their anti-inflammatory action, are the most powerful drugs. Besides this, β_2 agonists and methyl xanthine derivatives which all cause broncho dilation, and disodium chromoglycate which 'stabilises' mast cells, thereby preventing mediator release, all have proven beneficial in asthma patients (Ortega & Busse 1997).

Future treatment of asthma may as discussed above include anti-IL-5 mAb's. Celltech in corporation with Schering Plough have anti-IL-5 mAb in phase I clinical trial for treatment of asthma. However, treatment with monoclonal antibodies entails a number of drawbacks. First of all, the development and production costs for a safe mAB (e.g a humanised mAB) are very high, resulting in an expensive therapeutic product for the end user. Second, mABs have the disadvantageous characteristic seen from a patient point of view that they have to be administered with relatively short intervals. Third, by nature mABs exhibit a narrow specificity against one single epitope of the antigen. And, finally, mABs (even humanised) are immunogenic, leading to an increasingly fast inactivation of administered antibodies as treatment progresses over time.

Also use of antisense IL-5 oligonucleotides for antisense therapy has been suggested by the company Hybridon for the treatment of asthma, allergies and inflammation. However, the antisense technology has proven to be technically difficult and,

in fact, conclusive evidence of the feasibility of antisense therapy in humans has not yet been established.

Finally, WO 97/45448 (Bresagen Limited / Medvet Science) proposes the use of "modified and variant forms of IL-5 molecules capable of antagonising the activity of IL-5" in ameliorating, abating or otherwise reducing the aberrant effects caused by native or mutant forms of IL-5. The antagonizing effect is reported to be the result of the variant forms of IL-5 binding to the low affinity α chain of IL-5R but not to the high affinity receptors; in this way the variants compete with IL-5 for binding to its receptors without exerting the physiological effects of IL-5.

Other atopic diseases involving eosinophilic inflammation are treated with either the symptomatica mentioned for asthma or immune therapy (IT) using hyposensitization with allergen extracts. The latter type of treatment is known to be effective against allergies against one or a few antigens, whereas IT is not feasible in the treatment of multiple allergies. Furthermore, the time scale for obtaining clinical improvement in patients susceptible to treatment is very long for conventional IT.

Thus, in spite of existing and possible future therapies for chronic allergic diseases such as asthma, there is a definite need for alternative ways of treating and ameliorating this and other chronic allergic diseases.

OBJECT OF THE INVENTION

The object of the present invention is to provide novel therapies against chronic allergic conditions (such as asthma) characterized by eosinophilia. A further object is to develop an autovaccine against IL-5, in order to obtain a novel treatment

for asthma and for other pathological disorders involving chronic airway inflammation.

SUMMARY OF THE INVENTION

The T-cell derived cytokine IL-5 has, as mentioned above, a crucial role in orchestrating the eosinophilic response, affecting both the production, the localisation and the activation of eosinophils. As IL-5 has not otherwise been reported to have a central role in the development of a protective immune response, this particular cytokine is in the opinion of the inventors an attractive therapeutic target for the treatment of asthma.

The general aim according to the present invention is to decrease the pathogenic levels of eosinophils in the airways of the asthma patient by down-regulating of the IL-5 levels, since eosinophils depend on IL-5 for attraction and activation. The result of a decreased eosinophil number in the airway mucosa would be a concomitant decrease in the airway inflammation, corresponding to a clinical improvement in the asthmatic patient.

The potential effect of such an approach has already been demonstrated in studies using anti IL-5 monoclonal antibodies in animal models of airway inflammation, cf. the "PREAMBLE TO EXAMPLES".

This current invention, however, takes the results obtained through passive immunisation one step further by using the approach of generating an active immune response through the concept of autovaccination. To the best of the inventor's knowledge, such an approach has never been suggested before.

The advantage of treating asthmatics with an IL-5 autovaccine, as compared to current treatment with corticosteroids etc., is a reduction and/or elimination of side effects and most likely a better effect in terms of duration. When compared to anti-IL-5 mAbs, the effect of an induced polyclonal Ab response is expected to be superior to passively injected monoclonal immunoglobulins since the polyclonal response has a broader specificity. Improvements with respect to administration regimen are also expected (since effective autovaccines described herein typically would require a maximum of 2-6 administrations per year).

When compared to hyposensitization, the present invention offers the attractive aspect of being non-specific; this is especially relevant when dealing with multi-allergic patients.

Thus, in its broadest and most general scope, the present invention relates to a method for *in vivo* down-regulation of interleukin 5 (IL-5) activity in an animal, including a human being, the method comprising effecting presentation to the animal's immune system of an immunologically effective amount of

- at least one IL-5 polypeptide or subsequence thereof which has been formulated so that immunization of the animal with the IL-5 polypeptide or subsequence thereof induces production of antibodies against the IL-5 polypeptide, and/or
- at least one IL-5 analogue wherein is introduced at least one modification in the IL-5 amino acid sequence which has as a result that immunization of the animal with the analogue induces production of antibodies against the IL-5 polypeptide.

The most attractive aspect of this approach is that e.g. asthma can be controlled by periodic but not very frequent immunizations, in contrast to a therapeutic approach which involves administration of anti-IL-5 or molecules having a binding affinity to IL-5 analogous therewith. It is expected

that 1-4 annual injections with an immunogenic composition according to the invention will be sufficient to obtain the desired effect, whereas administration of other inhibitors of IL-5 activity does or will require daily, or at least weekly, administrations.

The invention also relates to IL-5 analogues as well as to nucleic acid fragments encoding a subset of these. Also immunogenic compositions comprising the analogues or the nucleic acid fragments are part of the invention.

The invention also relates to a method of identifying analogues of IL-5 as well as a method for preparing a composition comprising the IL-5 analogues.

LEGENDS TO THE FIGURES

Fig. 1: The amino acid sequence of the mature human IL-5 (SEQ ID NO: 1). The aligned murine sequence is included (SEQ ID NO: 12), but only positions that differ from the human sequence are displayed. The two "*"s indicate the missing N-terminal residues of the murine IL-5. The N-glycosylation positions are marked with double underlining, the O-glycosylated threonines of human IL-5 are given in italics, and the cysteines in bold.

Fig. 2: The dimer and monomer structures of human IL-5.
A: Dimer structure of hIL5. The structure has only been obtained for residues 5-112, which means that the O-glycosylation site at Thr3 is not included.
B: The same structure as in A, with the assignment of the helices (A-D and A'-D').
C: The monomer hIL-5 with the amino acid residues differing from the mIL-5 shown in light grey.

Fig. 3: The aligned mature human IL-5 (hIL-5) and murine IL-5 (mIL-5) amino acid sequences (SEQ ID NOs: 1 and 12) with indications of suitable substitution regions. The 4 α-helices A-D are surrounded by solid-line boxes, the β-sheets are double underlined and the positions of the two cysteines are marked with "▾". Identical residues in the two sequences are marked with "-" and non-identical residues with "*". Loop 1 spans between helices A and B, Loop 2 spans between helices B and C, and loop 3 spans between loops C and D. Amino acid sequences to be substituted with foreign T_H epitope containing peptides are marked in bold; one such sequence is surrounded by a dot-lined box because of residues overlapping with those substituted in a different construct. The amino acid sequences of 10 constructs (5 derived from human and 5 derived from murine IL-5) are set forth in SEQ ID NOs: 2-11 and 13-22.

DETAILED DISCLOSURE OF THE INVENTION

Definitions

In the following, a number of terms used in the present specification and claims will be defined and explained in detail in order to clarify the metes and bounds of the invention.

The terms "T-lymphocyte" and "T-cell" will be used interchangeably for lymphocytes of thymic origin which are responsible for various cell mediated immune responses as well as for helper activity in the humoral immune response. Likewise, the terms "B-lymphocyte" and "B-cell" will be used interchangeably for antibody-producing lymphocytes.

An "IL-5 polypeptide" is herein intended to denote polypeptides having the amino acid sequence of the above-discussed IL-5 proteins derived from humans and mice (or truncates thereof sharing a substantial amount of B-cell epitopes with intact IL-5), but also polypeptides having the amino acid sequence identical to xeno-analogues of these two proteins isolated from other species are embraced by the term. Also unglycosylated forms of IL-5 which are prepared in prokaryotic system are included within the boundaries of the term as are forms having varying glycosylation patterns due to the use of e.g. yeasts or other non-mammalian eukaryotic expression systems. It should, however, be noted that when using the term "an IL-5 polypeptide" it is intended that the polypeptide in question is normally non-immunogenic when presented to the animal to be treated. In other words, the IL-5 polypeptide is a self-protein or is a xeno-analogue of such a self-protein which will not normally give rise to an immune response against IL-5 of the animal in question.

An "IL-5 analogue" is an IL-5 polypeptide which has been subjected to changes in its primary structure. Such a change can e.g. be in the form of fusion of an IL-5 polypeptide to a suitable fusion partner (*i.e.* a change in primary structure exclusively involving C- and/or N-terminal additions of amino acid residues) and/or it can be in the form of insertions and/or deletions and/or substitutions in the IL-5 polypeptide's amino acid sequence. Also encompassed by the term are derivatized IL-5 molecules, cf. the discussion below of modifications of IL-5.

It should be noted that the use as a vaccine in a human of e.g. a canine analogue of human IL-5 can be imagined to produce the desired immunity against IL-5. Such use of an xeno-analogue for immunization is also considered to be an "IL-5 analogue" as defined above.

The term "polypeptide" is in the present context intended to mean both short peptides of from 2 to 10 amino acid residues, oligopeptides of from 11 to 100 amino acid residues, and polypeptides of more than 100 amino acid residues. Furthermore, the term is also intended to include proteins, *i.e.* functional biomolecules comprising at least one polypeptide; when comprising at least two polypeptides, these may form complexes, be covalently linked, or may be non-covalently linked. The polypeptide(s) in a protein can be glycosylated and/or lipidated and/or comprise prosthetic groups.

The term "subsequence" means any consecutive stretch of at least 3 amino acids or, when relevant, of at least 3 nucleotides, derived directly from a naturally occurring IL-5 amino acid sequence or nucleic acid sequence, respectively.

The term "animal" is in the present context in general intended to denote an animal species (preferably mammalian), such as *Homo sapiens*, *Canis domesticus*, etc. and not just one single animal. However, the term also denotes a population of such an animal species, since it is important that the individuals immunized according to the method of the invention all harbour substantially the same IL-5 allowing for immunization of the animals with the same immunogen(s). If, for instance, genetic variants of IL-5 exists in different human population it may be necessary to use different immunogens in these different populations in order to be able to break the autotolerance towards IL-5 in each population. It will be clear to the skilled person that an animal in the present context is a living being which has an immune system. It is preferred that the animal is a vertebrate, such as a mammal.

By the term "*in vivo* down-regulation of IL-5 activity" is herein meant reduction in the living organism of the number of interactions between IL-5 and its receptors (or between IL-5 and other possible biologically important binding partners for this

molecule). The down-regulation can be obtained by means of several mechanisms: Of these, simple interference with the active site in IL-5 by antibody binding is the most simple. However, it is also within the scope of the present invention that the antibody binding results in removal of IL-5 by scavenger cells (such as macrophages and other phagocytic cells).

The expression "effecting presentation ... to the immune system" is intended to denote that the animal's immune system is subjected to an immunogenic challenge in a controlled manner. As will appear from the disclosure below, such challenge of the immune system can be effected in a number of ways of which the most important are vaccination with polypeptide containing "pharmaccines" (*i.e.* a vaccine which is administered to treat or ameliorate ongoing disease) or nucleic acid "pharmaccine" vaccination. The important result to achieve is that immune competent cells in the animal are confronted with the antigen in an immunologically effective manner, whereas the precise mode of achieving this result is of less importance to the inventive idea underlying the present invention.

The term "immunogenically effective amount" has its usual meaning in the art, *i.e.* an amount of an immunogen which is capable of inducing an immune response which significantly engages pathogenic agents which share immunological features with the immunogen.

When using the expression that the IL-5 has been "modified" is herein meant a chemical modification of the polypeptide which constitutes the backbone of IL-5. Such a modification can e.g. be derivatization (e.g. alkylation, acylation, esterification etc.) of certain amino acid residues in the IL-5 sequence, but as will be appreciated from the disclosure below, the preferred modifications comprise changes of (or additions to) the primary structure of the IL-5 amino acid sequence.

When discussing "autotolerance towards IL-5" it is understood that since IL-5 is a self-protein in the population to be vaccinated, normal individuals in the population do not mount an immune response against IL-5; it cannot be excluded, though, that occasional individuals in an animal population might be able to produce antibodies against native IL-5, e.g. as part of an autoimmune disorder. At any rate, an animal will normally only be autotolerant towards its own IL-5, but it cannot be excluded that IL-5 analogues derived from other animal species or from a population having a different IL-5 phenotype would also be tolerated by said animal.

A "foreign T-cell epitope" (or: "foreign T-lymphocyte epitope") is a peptide which is able to bind to an MHC molecule and which stimulates T-cells in an animal species. Preferred foreign T-cell epitopes in the invention are "promiscuous" epitopes, *i.e.* epitopes which bind to a substantial fraction of a particular class of MHC molecules in an animal species or population. Only a very limited number of such promiscuous T-cell epitopes are known, and they will be discussed in detail below. It should be noted that in order for the immunogens which are used according to the present invention to be effective in as large a fraction of an animal population as possible, it may be necessary to 1) insert several foreign T-cell epitopes in the same IL-5 analogue or 2) prepare several IL-5 analogues wherein each analogue has a different promiscuous epitope inserted. It should be noted also that the concept of foreign T-cell epitopes also encompasses use of cryptic T-cell epitopes, *i.e.* epitopes which are derived from a self-protein and which only exerts immunogenic behaviour when existing in isolated form without being part of the self-protein in question.

A "foreign T helper lymphocyte epitope" (a foreign T_H epitope) is a foreign T cell epitope which binds an MHC Class Class II molecule and can be presented on the surface of an antigen presenting cell (APC) bound to the MHC Class II molecule.

A "functional part" of a (bio)molecule is in the present context intended to mean the part of the molecule which is responsible for at least one of the biochemical or physiological effects exerted by the molecule. It is well-known in the art that many enzymes and other effector molecules have an active site which is responsible for the effects exerted by the molecule in question. Other parts of the molecule may serve a stabilizing or solubility enhancing purpose and can therefore be left out if these purposes are not of relevance in the context of a certain embodiment of the present invention. For instance it is possible to use certain other cytokines as a modifying moiety in IL-5 (cf. the detailed discussion below), and in such a case, the issue of stability may be irrelevant since the coupling to IL-5 provides the stability necessary.

The term "adjuvant" has its usual meaning in the art of vaccine technology, *i.e.* a substance or a composition of matter which is 1) not in itself capable of mounting a specific immune response against the immunogen of the vaccine, but which is 2) nevertheless capable of enhancing the immune response against the immunogen. Or, in other words, vaccination with the adjuvant alone does not provide an immune response against the immunogen, vaccination with the immunogen may or may not give rise to an immune response against the immunogen, but the combined vaccination with immunogen and adjuvant induces an immune response against the immunogen which is stronger than that induced by the immunogen alone.

"Targeting" of a molecule is in the present context intended to denote the situation where a molecule upon introduction in the animal will appear preferentially in certain tissue(s) or will be preferentially associated with certain cells or cell types. The effect can be accomplished in a number of ways including formulation of the molecule in composition facilitating targeting or by introduction in the molecule of groups which

facilitates targeting. These issues will be discussed in detail below.

"Stimulation of the immune system" means that a substance or composition of matter exhibits a general, non-specific immunostimulatory effect. A number of adjuvants and putative adjuvants (such as certain cytokines) share the ability to stimulate the immune system. The result of using an immunostimulating agent is an increased "alertness" of the immune system meaning that simultaneous or subsequent immunization with an immunogen induces a significantly more effective immune response compared to isolated use of the immunogen

Preferred embodiments of IL-5 activity down-regulation

It is preferred that the IL-5 polypeptide used as an immunogen in the method of the invention is a modified molecule wherein at least one change is present in the IL-5 amino acid sequence, since the chances of obtaining the all-important breaking of autotolerance towards IL-5 is greatly facilitated that way. It should be noted that this does not exclude the possibility of using such a modified IL-5 in formulations which further facilitate the breaking of autotolerance against IL-5, e.g. formulations containing certain adjuvants discussed in detail below.

It has been shown (in Dalum I *et al.*, 1996, J. Immunol. **157**: 4796-4804) that potentially self-reactive B-lymphocytes recognizing self-proteins are physiologically present in normal individuals. However, in order for these B-lymphocytes to be induced to actually produce antibodies reactive with the relevant self-proteins, assistance is needed from cytokine producing T-helper lymphocytes (T_H-cells or T_H-lymphocytes). Normally this help is not provided because T-lymphocytes in general do not recognize T-cell epitopes derived from self-

proteins when presented by antigen presenting cells (APCs). However, by providing an element of "foreignness" in a self-protein (*i.e.* by introducing an immunologically significant modification), T-cells recognizing the foreign element are activated upon recognizing the foreign epitope on an APC (such as, initially, a mononuclear cell). Polyclonal B-lymphocytes (which are also specialised APCs) capable of recognising self-epitopes on the modified self-protein also internalise the antigen and subsequently presents the foreign T-cell epitope(s) thereof, and the activated T-lymphocytes subsequently provide cytokine help to these self-reactive polyclonal B-lymphocytes. Since the antibodies produced by these polyclonal B-lymphocytes are reactive with different epitopes on the modified polypeptide, including those which are also present in the native polypeptide, an antibody cross-reactive with the non-modified self-protein is induced. In conclusion, the T-lymphocytes can be led to act as if the population of polyclonal B-lymphocytes have recognised an entirely foreign antigen, whereas in fact only the inserted epitope(s) is/are foreign to the host. In this way, antibodies capable of *cross-reacting* with non-modified self-antigens are induced.

Several ways of modifying a peptide self-antigen in order to obtain breaking of autotolerance are known in the art. Hence, according to the invention, the modification can include that

- at least one foreign T-cell epitope is introduced, and/or
- at least one first moiety is introduced which effects targeting of the modified molecule to an antigen presenting cell (APC), and/or
- at least one second moiety is introduced which stimulates the immune system, and/or
- at least one third moiety is introduced which optimizes presentation of the modified IL-5 polypeptide to the immune system.

However, all these modifications should be carried out while maintaining a substantial fraction of the original B-lymphocyte epitopes in IL-5, since the B-lymphocyte recognition of the native molecule is thereby enhanced.

In one preferred embodiment, side groups (in the form of foreign T-cell epitopes or the above-mentioned first, second and third moieties) are covalently or non-covalently introduced. This is intended to mean that stretches of amino acid residues derived from IL-5 are derivatized without altering the primary amino acid sequence, or at least without introducing changes in the peptide bonds between the individual amino acids in the chain.

An alternative, and preferred, embodiment utilises amino acid substitution and/or deletion and/or insertion and/or addition (which may be effected by recombinant means or by means of peptide synthesis; modifications which involves longer stretches of amino acids can give rise to fusion polypeptides). One especially preferred version of this embodiment is the technique described in WO 95/05849, which discloses a method for down-regulating self-proteins by immunising with analogues of the self-proteins wherein a number of amino acid sequence(s) has been substituted with a corresponding number of amino acid sequence(s) which each comprise a foreign immunodominant T-cell epitope, while at the same time maintaining the overall tertiary structure of the self-protein in the analogue. For the purposes of the present invention, it is however sufficient if the modification (be it an amino acid insertion, addition, deletion or substitution) gives rise to a foreign T-cell epitope and at the same time preserves a substantial number of the B-cell epitopes in IL-5. However, in order to obtain maximum efficacy of the immune response induced, it is preferred that the overall tertiary structure of IL-5 is maintained in the modified molecule.

The following formula describes the IL-5 constructs generally covered by the invention:

$$(MOD_1)_{s1}(IL\text{-}5_{e1})_{n1}(MOD_2)_{s2}(IL\text{-}5_{e2})_{n2}\ldots.(MOD_x)_{sx}(IL\text{-}5_{ex})_{nx} \qquad (I)$$

-where $IL\text{-}5_{e1}$-$IL\text{-}5_{ex}$ are x B-cell epitope containing subsequences of IL-5 which independently are identical or non-identical and which may contain or not contain foreign side groups, x is an ~~integer ≥ 3, n1-nx are x integers ≥ 0 (at least one is ≥ 1)~~, MOD_1-MOD_x are x modifications introduced between the preserved B-cell epitopes, and s_1-s_x are x integers ≥ 0 (at least one is ≥ 1 if no side groups are introduced in the $IL\text{-}5_e$ sequences). Thus, given the general functional restraints on the immunogenicity of the constructs, the invention allows for all kinds of permutations of the original IL-5 sequence, and all kinds of modifications therein. Thus, included in the invention are modified IL-5 obtained by omission of parts of the IL-5 sequence which e.g. exhibit adverse effects *in vivo* or omission of parts which could give rise to undesired immunological reactions.

Maintenance of a substantial fraction of B-cell epitopes or even the overall tertiary structure of a protein which is subjected to modification as described herein can be achieved in several ways. One is simply to prepare a polyclonal antiserum directed against IL-5 (e.g. an antiserum prepared in a rabbit) and thereafter use this antiserum as a test reagent (e.g. in a competitive ELISA) against the modified proteins which are produced. Modified versions (analogues) which react to the same extent with the antiserum as does IL-5 must be regarded as having the same overall tertiary structure as IL-5 whereas analogues exhibiting a limited (but still significant and specific) reactivity with such an antiserum are regarded as having maintained a substantial fraction of the original B-cell epitopes.

Alternatively, a selection of monoclonal antibodies reactive with distinct epitopes on IL-5 can be prepared and used as a test panel. This approach has the advantage of allowing 1) an epitope mapping of IL-5 and 2) a mapping of the epitopes which are maintained in the analogues prepared.

Of course, a third approach would be to resolve the 3-dimensional structure of IL-5 or of a biologically active truncate thereof (cf. above) and compare this to the resolved three-dimensional structure of the analogues prepared. Three-dimensional structure can be resolved by the aid of X-ray diffraction studies and NMR-spectroscopy. Further information relating to the tertiary structure can to some extent be obtained from circular dichroism studies which have the advantage of merely requiring the polypeptide in pure form (whereas X-ray diffraction requires the provision of crystallized polypeptide and NMR requires the provision of isotopic variants of the polypeptide) in order to provide useful information about the tertiary structure of a given molecule. However, ultimately X-ray diffraction and/or NMR are necessary to obtain conclusive data since circular dichroism can only provide indirect evidence of correct 3-dimensional structure via information of secondary structure elements.

One preferred embodiment of the invention utilises multiple presentations of B-lymphocyte epitopes of IL-5 (*i.e.* formula I wherein at least one B-cell epitope is present in two positions). This effect can be achieved in various ways, e.g. by simply preparing fusion polypeptides comprising the structure $(IL\text{-}5)_m$, where m is an integer ≥ 2 and then introduce the modifications discussed herein in at least one of the IL-5 sequences, or alternatively, inserted between at least two of the IL-5 amino acid sequences. It is preferred that the modifications introduced includes at least one duplication of a B-lymphocyte epitope and/or the introduction of a hapten.

As mentioned above, the introduction of a foreign T-cell epitope can be accomplished by introduction of at least one amino acid insertion, addition, deletion, or substitution. Of course, the normal situation will be the introduction of more than one change in the amino acid sequence (e.g. insertion of or substition by a complete T-cell epitope) but the important goal to reach is that the IL-5 analogue, when processed by an antigen presenting cell (APC), will give rise to such a foreign immunodominant T-cell epitope being presented in context of an MCH Class II molecule on the surface of the APC. Thus, if the IL-5 amino acid sequence in appropriate positions comprises a number of amino acid residues which can also be found in a foreign T_H epitope then the introduction of a foreign T_H epitope can be accomplished by providing the remaining amino acids of the foreign epitope by means of amino acid insertion, addition, deletion and substitution. In other words, it is not necessary to introduce a complete T_H epitope by insertion or substitution.

It is preferred that the number of amino acid insertions, deletions, substitutions or additions is at least 2, such as 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, and 25 insertions, substitutions, additions or deletions. It is furthermore preferred that the number of amino acid insertions, substitutions, additions or deletions is not in excess of 150, such as at most 100, at most 90, at most 80, and at most 70. It is especially preferred that the number of substitutions, insertions, deletions, or additions does not exceed 60, and in particular the number should not exceed 50 or even 40. Most preferred is a number of not more than 30. With respect to amino acid additions, it should be noted that these, when the resulting construct is in the form of a fusion polypeptide, is often considerably higher than 150.

Preferred embodiments of the invention includes modification by introducing at least one foreign immunodominant T_H epitope. It will be understood that the question of immune dominance of a T_H

epitope depends on the animal species in question. As used herein, the term "immunodominance" simply refers to epitopes which in the vaccinated individual gives rise to a significant immune response, but it is a well-known fact that a T_H epitope which is immunodominant in one individual is not necessarily immunodominant in another individual of the same species, even though it may be capable of binding MHC-II molecules in the latter individual.

Another important point is the issue of MHC restriction of T_H epitopes. In general, naturally occurring T_H epitopes are MHC restricted, *i.e.* a certain peptide constituting a T_H epitope will only bind effectively to a subset of MHC Class II molecules. This in turn has the effect that in most cases the use of one specific T_H epitope will result in a vaccine component which is effective in a fraction of the population only, and depending on the size of that fraction, it can be necessary to include more T_H epitopes in the same molecule, or alternatively prepare a multi-component vaccine wherein the components are IL-5 variants which are distinguished from each other by the nature of the T_H epitope introduced.

If the MHC restriction of the T-cells used is completely unknown (for instance in a situation where the vaccinated animal has a poorly defined MHC composition), the fraction of the animal population covered by a specific vaccine composition can be determined by means of the following formula

$$f_{population} = 1 - \prod_{i=1}^{n} (1 - p_i) \qquad \text{(II)}$$

-where p_i is the frequency in the population of responders to the i^{th} foreign T-cell epitope present in the vaccine composition, and n is the total number of foreign T-cell epitopes in the vaccine composition. Thus, a vaccine composition

containing 3 foreign T-cell epitopes having response frequencies in the population of 0.8, 0.7, and 0.6, respectively, would give

$$1 - 0.2 \times 0.3 \times 0.4 = 0.976$$

-*i.e.* 97.6 percent of the population will statistically mount an MHC-II mediated response to the vaccine.

The above formula does not apply in situations where a more or less precise MHC restriction pattern of the peptides used is known. If, for instance a certain peptide only binds the human MHC-II molecules encoded by HLA-DR alleles DR1, DR3, DR5, and DR7, then the use of this peptide together with another peptide which binds the remaining MHC-II molecules encoded by HLA-DR alleles will accomplish 100% coverage in the population in question. Likewise, if the second peptide only binds DR3 and DR5, the addition of this peptide will not increase the coverage at all. If one bases the calculation of population response purely on MHC restriction of T-cell epitopes in the vaccine, the fraction of the population covered by a specific vaccine composition can be determined by means of the following formula:

$$f_{population} = 1 - \prod_{j=1}^{3} (1 - \varphi_j)^2 \qquad \text{(III)}$$

-wherein φ_j is the sum of frequencies in the population of allelic haplotypes encoding MHC molecules which bind any one of the T-cell epitopes in the vaccine and which belong to the j^{th} of the 3 known HLA loci (DP, DR and DQ); in practice, it is first determined which MHC molecules will recognize each T-cell epitope in the vaccine and thereafter these MHC molecules are listed by type (DP, DR and DQ) - then, the individual frequencies of the different listed allelic haplotypes are summed for each type, thereby yielding φ_1, φ_2, and φ_3.

It may occur that the value p_i in formula II exceeds the corresponding theoretical value π_i:

$$\pi_i = 1 - \prod_{j=1}^{3} (1 - \nu_j)^2 \qquad \text{(IV)}$$

-wherein υ_j is the sum of frequencies in the population of allelic haplotypes encoding MHC molecules which bind the i^{th} T-cell epitope in the vaccine and which belong to the j^{th} of the 3 known HLA loci (DP, DR and DQ). This means that in $1-\pi_i$ of the population is a frequency of responders of $f_{residual\_i} = (p_i-\pi_i)/(1-\pi_i)$. Therefore, formula III can be adjusted so as to yield formula V:

$$f_{population} = 1 - \prod_{j=1}^{3} (1 - \varphi_j)^2 + \left(1 - \prod_{i=1}^{n} (1 - f_{residual\_i})\right) \qquad \text{(V)}$$

-where the term $1-f_{residual-i}$ is set to zero if negative. It should be noted that formula V requires that all epitopes have been haplotype mapped against identical sets of haplotypes.

Therefore, when selecting T-cell epitopes to be introduced in the IL-5 analogue, it is important to include all knowledge of the epitopes which is available: 1) The frequency of responders in the population to each epitope, 2) MHC restriction data, and 3) frequency in the population of the relevant haplotypes.

There exists a number of naturally occurring "promiscuous" T-cell epitopes which are active in a large proportion of individuals of an animal species or an animal population and these are preferably introduced in the vaccine thereby reducing the need for a very large number of different IL-5 analogues in the same vaccine.

The promiscuous epitope can according to the invention be a naturally occurring human T-cell epitope such as epitopes from

tetanus toxoid (e.g. the P2 and P30 epitopes), diphtheria toxoid, Influenza virus hemagluttinin (HA), and *P. falciparum* CS antigen.

Over the years a number of other promiscuous T-cell epitopes have been identified. Especially peptides capable of binding a large proportion of HLA-DR molecules encoded by the different HLA-DR alleles have been identified and these are all possible T-cell epitopes to be introduced in the IL-5 analogues used according to the present invention. Cf. also the epitopes discussed in the following references which are hereby all incorporated by reference herein: WO 98/23635 (Frazer IH *et al.*, assigned to The University of Queensland); Southwood S *et. al*, 1998, J. Immunol. **160**: 3363-3373; Sinigaglia F *et al.*, 1988, Nature **336**: 778-780; Chicz RM *et al.*, 1993, J. Exp. Med **178**: 27-47; Hammer J *et al.*, 1993, Cell **74**: 197-203; and Falk K *et al.*, 1994, Immunogenetics **39**: 230-242. The latter reference also deals with HLA-DQ and -DP ligands. All epitopes listed in these 5 references are relevant as candidate natural epitopes to be used in the present invention, as are epitopes which share common motifs with these.

Alternatively, the epitope can be any artificial T-cell epitope which is capable of binding a large proportion of MHC Class II molecues. In this context the pan DR epitope peptides ("PADRE") described in WO 95/07707 and in the corresponding paper Alexander J *et al.*, 1994, Immunity **1**: 751-761 (both disclosures are incorporated by reference herein) are interesting candidates for epitopes to be used according to the present invention. It should be noted that the most effective PADRE peptides disclosed in these papers carry D-amino acids in the C- and N-termini in order to improve stability when administered. However, the present invention primarily aims at incorporating the relevant epitopes as part of the modified IL-5 which should then subsequently be broken down enzymatically inside the lysosomal compartment of APCs to allow subsequent presentation in the

context of an MHC-II molecule and therefore it is not expedient to incorporate D-amino acids in the epitopes used in the present invention.

One especially preferred PADRE peptide is the one having the amino acid sequence AKFVAAWTLKAAA or an immunologically effective subsequence thereof. This, and other epitopes having the same lack of MHC restriction are preferred T-cell epitopes which should be present in the IL-5 analogues used in the inventive method. Such super-promiscuous epitopes will allow for the most simple embodiments of the invention wherein only one single modified IL-5 is presented to the vaccinated animal's immune system.

As mentioned above, the modification of IL-5 can also include the introduction of a first moiety which targets the modified IL-5 to an APC or a B-lymphocyte. For instance, the first moiety can be a specific binding partner for a B-lymphocyte specific surface antigen or for an APC specific surface antigen. Many such specific surface antigens are known in the art. For instance, the moiety can be a carbohydrate for which there is a receptor on the B-lymphocyte or on the APC (e.g. mannan or mannose). Alternatively, the second moiety can be a hapten. Also an antibody fragment which specifically recognizes a surface molecule on APCs or lymphocytes can be used as a first moiety (the surface molecule can e.g. be an FCγ receptor of macrophages and monocytes, such as FCγRI or, alternatively any other specific surface marker such as CD40 or CTLA-4). It should be noted that all these exemplary targeting molecules can be used as part of an adjuvant also, cf. below.

As an alternative or supplement to targeting the modified IL-5 polypeptide to a certain cell type in order to achieve an enhanced immune response, it is possible to increase the level of responsiveness of the immune system by including the above-mentioned second moiety which stimulates the immune system.

Typical examples of such second moieties are cytokines, and heat-shock proteins or molecular chaperones, as well as effective parts thereof.

Suitable cytokines to be used according to the invention are those which will normally also function as adjuvants in a vaccine composition, *i.e.* for instance interferon γ (IFN-γ), Flt3L, interleukin 1 (IL-1), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 6 (IL-6), interleukin 12 (IL-12), interleukin 13 (IL-13), interleukin 15 (IL-15), and granulocyte-macrophage colony stimulating factor (GM-CSF); alternatively, the functional part of the cytokine molecule may suffice as the second moiety. With respect to the use of such cytokines as adjuvant substances, cf. the discussion below. It should be noted that use of both IL-4 and IL-13 should be exercised very carefully, if at all, as both molecules are known as key effector molecules in the pathophysiology of atopy and asthma.

According to the invention, suitable heat-shock proteins or molecular chaperones used as the second moiety can be HSP70, HSP90, HSC70, GRP94 (also known as gp96, cf. Wearsch PA *et al.* 1998, Biochemistry **37**: 5709-19), and CRT (calreticulin).

Alternatively, the second moiety can be a toxin, such as listeriolycin (LLO), lipid A and heat-labile enterotoxin. Also, a number of mycobacterial derivatives such as MDP (muramyl dipeptide) and the trehalose diesters TDM and TDE are interesting possibilities.

Also the possibility of introducing a third moiety which enhances the presentation of the modified IL-5 to the immune system is an important embodiment of the invention. The art has shown several examples of this principle. For instance, it is known that the palmitoyl lipidation anchor in the *Borrelia burgdorferi* protein OspA can be utilised so as to provide self-adjuvating polypeptides (cf. e.g. WO 96/40718). It seems that

the lipidated proteins form up micelle-like structures with a core consisting of the lipidation anchor parts of the polypeptides and the remaining parts of the molecule protruding therefrom, resulting in multiple presentations of the antigenic determinants. Hence, the use of this and related approaches using different lipidation anchors (e.g. a myristyl group, a myristyl group, a farnesyl group, a geranyl-geranyl group, a GPI-anchor, and an N-acyl diglyceride group) are preferred embodiments of the invention, especially since the provision of such a lipidation anchor in a recombinantly produced protein is fairly straightforward and merely requires use of e.g. a naturally occurring signal sequence as a fusion partner for the modified IL-5 polypeptide. Another possibility is use of the C3d fragment of complement factor C3 or C3 itself (cf. Dempsey et *al.*, 1996, Science **271**, 348-350 and Lou & Kohler, 1998, Nature Biotechnology **16**, 458-462).

An alternative embodiment of the invention which also results in the preferred presentation of multiple (e.g. at least 2) copies of the important epitopic regions of IL-5 to the immune system is the covalent or non-covalent coupling of IL-5, subsequence or variants thereof to certain carrier molecules. For instance, polymers can be used, e.g. carbohydrates such as dextran, cf. e.g. Lees A *et al.*, 1994, Vaccine **12**: 1160-1166; Lees A *et al.*, 1990, J Immunol. **145**: 3594-3600, but also mannose and mannan are useful alternatives. Integral membrane proteins from e.g. *E. coli* and other bacteria are also useful conjugation partners. The traditional carrier molecules such as keyhole limpet hemocyanin (KLH), tetanus toxoid, diphtheria toxoid, and bovine serum albumin (BSA) are also preferred and useful conjugation partners.

Certain areas of native IL-5 are believed to be superiorly suited for performing modifications. It is predicted that modifications in at least one of loops 1-3 or in the amino acid residues C-terminal to helix D (said loops and said helix D

corresponding to those shown in Fig. 3 for human and murine IL-5) will be most likely to produce the desired constructs and vaccination results. Considerations underlying these chosen areas are a) preservation of known and predicted B-cell epitopes, b) preservation of tertiary and quaternary structures etc, cf. also the discussion in the preamble to the examples. At any rate, as discussed above, it is fairly easy to screen a set of modified IL-5 molecules which have all been subjected to introduction of a T-cell epitope in different locations.

Since the most preferred embodiments of the present invention involves down-regulation of human IL-5, it is consequently preferred that the IL-5 polypeptide discussed above is a human IL-5 polypeptide. In this embodiment, it is especially preferred that the human IL-5 polypeptide has been modified by substituting at least one amino acid sequence in SEQ ID NO: 1 with at least one amino acid sequence of equal or different length and containing a foreign T_H epitope, wherein substituted amino acid residues are selected from the group consisting of residues 87-90, residues 32-43, residues 59-64, residues 86-91, and residues 110-113. The rationale behind such constructs is discussed in detail in the examples.

Formulation of IL-5 and modified IL-5 polypeptides

When effecting presentation of the IL-5 polypeptide or the modified IL-5 polypeptide to an animal's immune system by means of administration thereof to the animal, the formulation of the polypeptide follows the principles generally acknowledged in the art.

Preparation of vaccines which contain peptide sequences as active ingredients is generally well understood in the art, as exemplified by U.S. Patents 4,608,251; 4,601,903; 4,599,231; 4,599,230; 4,596,792; and 4,578,770, all incorporated herein by reference. Typically, such vaccines are prepared as injectables

either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified. The active immunogenic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants which enhance the effectiveness of the vaccines; cf. the detailed discussion of adjuvants below.

The vaccines are conventionally administered parenterally, by injection, for example, either subcutaneously, intracutaneously, intradermally, subdermally or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral, buccal, sublinqual, intraperitoneal, intravaginal, anal, epidural, spinal, and intracranial formulations. For suppositories, traditional binders and carriers may include, for example, polyalkalene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10-95% of active ingredient, preferably 25-70%. For oral formulations, cholera toxin is an interesting formulation partner (and also a possible conjugation partner).

The polypeptides may be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts include acid

addition salts (formed with the free amino groups of the peptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective and immunogenic. The quantity to be administered depends on the subject to be treated, including, e.g., the capacity of the individual's immune system to mount an immune response, and the degree of protection desired. Suitable dosage ranges are of the order of several hundred micrograms active ingredient per vaccination with a preferred range from about 0.1 µg to 2,000 µg (even though higher amounts in the 1-10 mg range are contemplated), such as in the range from about 0.5 µg to 1,000 µg, preferably in the range from 1 µg to 500 µg and especially in the range from about 10 µg to 100 µg. Suitable regimens for initial administration and booster shots are also variable but are typified by an initial administration followed by subsequent inoculations or other administrations.

The manner of application may be varied widely. Any of the conventional methods for administration of a vaccine are applicable. These include oral application on a solid physiologically acceptable base or in a physiologically acceptable dispersion, parenterally, by injection or the like. The dosage of the vaccine will depend on the route of administration and will vary according to the age of the person to be vaccinated and the formulation of the antigen.

Some of the polypeptides of the vaccine are sufficiently immunogenic in a vaccine, but for some of the others the immune response will be enhanced if the vaccine further comprises an adjuvant substance.

Various methods of achieving adjuvant effect for the vaccine are known. General principles and methods are detailed in "The Theory and Practical Application of Adjuvants", 1995, Duncan E.S. Stewart-Tull (ed.), John Wiley & Sons Ltd, ISBN 0-471-95170-6, and also in "Vaccines: New Generationn Immunological Adjuvants", 1995, Gregoriadis G *et al.* (eds.), Plenum Press, New York, ISBN 0-306-45283-9, both of which are hereby incorporated by reference herein.

It is especially preferred to use an adjuvant which can be demonstrated to facilitate breaking of the autotolerance to autoantigens; in fact, this is essential in cases where unmodified IL-5 is used as the active ingredient in the autovaccine. Non-limiting examples of suitable adjuvants are selected from the group consisting of an immune targeting adjuvant; an immune modulating adjuvant such as a toxin, a cytokine, and a mycobacterial derivative; an oil formulation; a polymer; a micelle forming adjuvant; a saponin; an immunostimulating complex matrix (ISCOM matrix); a particle; DDA; aluminium adjuvants; DNA adjuvants; γ-inulin; and an encapsulating adjuvant. In general it should be noted that the disclosures above which relate to compounds and agents useful as first, second and third moieties in the analogues also refer *mutatis mutandis* to their use in the adjuvant of a vaccine of the invention.

The application of adjuvants include use of agents such as aluminum hydroxide or phosphate (alum), commonly used as 0.05 to 0.1 percent solution in buffered saline, admixture with synthetic polymers of sugars (e.g. Carbopol®) used as 0.25 percent solution, aggregation of the protein in the vaccine by

heat treatment with temperatures ranging between 70° to 101°C for 30 second to 2 minute periods respectively and also aggregation by means of cross-linking agents are possible. Aggregation by reactivation with pepsin treated antibodies (Fab fragments) to albumin, mixture with bacterial cells such as *C. parvum* or endotoxins or lipopolysaccharide components of gram-negative bacteria, emulsion in physiologically acceptable oil vehicles such as mannide mono-oleate (Aracel A) or emulsion with 20 percent solution of a perfluorocarbon (Fluosol-DA) used as a block substitute may also be employed. Admixture with oils such as squalene and IFA is also preferred.

According to the invention DDA (dimethyldioctadecylammonium bromide) is an interesting candidate for an adjuvant as is DNA and γ-inulin, but also Freund's complete and incomplete adjuvants as well as *quillaja* saponins such as QuilA and QS21 are interesting as is RIBI. Further possibilities are monophosphoryl lipid A (MPL), the above mentioned C3 and C3d, and muramyl dipeptide (MDP).

Liposome formulations are also known to confer adjuvant effects, and therefore liposome adjuvants are preferred according to the invention.

Also immunostimulating complex matrix type (ISCOM® matrix) adjuvants are preferred choices according to the invention, especially since it has been shown that this type of adjuvants are capable of up-regulating MHC Class II expression by APCs. An ISCOM® matrix consists of (optionally fractionated) saponins (triterpenoids) from *Quillaja saponaria*, cholesterol, and phospholipid. When admixed with the immunogenic protein, the resulting particulate formulation is what is known as an ISCOM particle where the saponin constitutes 60-70% w/w, the cholesterol and phospholipid 10-15% w/w, and the protein 10-15% w/w. Details relating to composition and use of immunostimulating complexes can e.g. be found in the above-

mentioned text-books dealing with adjuvants, but also Morein B *et al.*, 1995, Clin. Immunother. **3**: 461-475 as well as Barr IG and Mitchell GF, 1996, Immunol. and Cell Biol. **74**: 8-25 (both incorporated by reference herein) provide useful instructions for the preparation of complete immunostimulating complexes.

Another highly interesting (and thus, preferred) possibility of achieving adjuvant effect is to employ the technique described in Gosselin et al., 1992 (which is hereby incorporated by reference herein). In brief, the presentation of a relevant antigen such as an antigen of the present invention can be enhanced by conjugating the antigen to antibodies (or antigen binding antibody fragments) against the Fcγ receptors on monocytes/macrophages. Especially conjugates between antigen and anti-FcγRI have been demonstrated to enhance immunogenicity for the purposes of vaccination.

Other possibilities involve the use of the targeting and immune modulating substances (*i.a.* cytokines) mentioned above as candidates for the first and second moieties in the modified versions of IL-5. In this connection, also synthetic inducers of cytokines like poly I:C are possibilities.

Suitable mycobacterial derivatives are selected from the group consisting of muramyl dipeptide, complete Freund's adjuvant, RIBI, and a diester of trehalose such as TDM and TDE.

Suitable immune targeting adjuvants are selected from the group consisting of CD40 ligand and CD40 antibodies or specifically binding fragments thereof (cf. the discussion above), mannose, a Fab fragment, and CTLA-4.

Suitable polymer adjuvants are selected from the group consisting of a carbohydrate such as dextran, PEG, starch, mannan, and mannose; a plastic polymer such as; and latex such as latex beads.

Yet another interesting way of modulating an immune response is to include the IL-5 immunogen (optionally together with adjuvants and pharmaceutically acceptable carriers and vehicles) in a "virtual lymph node" (VLN) (a proprietary medical device developed by ImmunoTherapy, Inc., 360 Lexington Avenue, New York, NY 10017-6501). The VLN (a thin tubular device) mimics the structrue and function of a lymph node. Insertion of a VLN under the skin creates a site of sterile inflammation with an upsurge of cytokines and chemokines. T- and B-cells as well as APCs rapidly respond to the danger signals, home to the inflamed site and accumulate inside the porous matrix of the VLN. It has been shown that the necessary antigen dose required to mount an immune response to an antigen is reduced when using the VLN and that immune protection conferred by vaccination using a VLN surpassed conventional immunization using Ribi as an adjuvant. The technology is *i.a.* described briefly in Gelber C *et al.*, 1998, "Elicitation of Robust Cellular and Humoral Immune Responses to Small Amounts of Immunogens Using a Novel Medical Device Designated the Virtual Lymph Node", in: "From the Laboratory to the Clinic, Book of Abstracts, October 12th - 15th 1998, Seascape Resort, Aptos, California".

It is expected that the vaccine should be administered at least once a year, such as at least 1, 2, 3, 4, 5, 6, and 12 times a year. More specifically, 1-12 times per year is expected, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 times a year to an individual in need thereof. It has previously been shown that the memory immunity induced by the use of the preferred autovaccines according to the invention is not permanent, and therefor the immune system needs to be periodically challenged with the analogues.

Due to genetic variation, different individuals may react with immune responses of varying strength to the same polypeptide. Therefore, the vaccine according to the invention may comprise several different polypeptides in order to increase the immune

response, cf. also the discussion above concerning the choice of foreign T-cell epitope introductions. The vaccine may comprise two or more polypeptides, where all of the polypeptides are as defined above.

The vaccine may consequently comprise 3-20 different modified or unmodified polypeptides, such as 3-10 different polypeptides. However, normally the number of polypeptides will be sought kept to a minimum such as 1 or 2 polypeptides.

Nucleic acid vaccination

As an alternative to classic administration of a peptide-based vaccine, the technology of nucleic acid vaccination (also known as "nucleic acid immunisation", "genetic immunisation", and "gene immunisation") offers a number of attractive features.

First, in contrast to the traditional vaccine approach, nucleic acid vaccination does not require resource consuming large-scale production of the immunogenic agent (e.g. in the form of industrial scale fermentation of microorganisms producing modified IL-5). Furthermore, there is no need to device purification and refolding schemes for the immunogen. And finally, since nucleic acid vaccination relies on the biochemical apparatus of the vaccinated individual in order to produce the expression product of the nucleic acid introduced, the optimum posttranslational processing of the expression product is expected to occur; this is especially important in the case of autovaccination, since, as mentioned above, a significant fraction of the original IL-5 B-cell epitopes should be preserved in the modified molecule, and since B-cell epitopes in principle can be constituted by parts of any (bio)molecule (e.g. carbohydrate, lipid, protein etc.). Therefore, native glycosylation and lipidation patterns of the immunogen may very well be of importance for the overall immunogenicity and this is

expected to be ensured by having the host producing the immunogen.

Hence, a preferred embodiment of the invention comprises effecting presentation of modified IL-5 to the immune system by introducing nucleic acid(s) encoding the modified IL-5 into the animal's cells and thereby obtaining *in vivo* expression by the cells of the nucleic acid(s) introduced.

In this embodiment, the introduced nucleic acid is preferably DNA which can be in the form of naked DNA, DNA formulated with charged or uncharged lipids, DNA formulated in liposomes, DNA included in a viral vector, DNA formulated with a transfection-facilitating protein or polypeptide, DNA formulated with a targeting protein or polypeptide, DNA formulated with Calcium precipitating agents, DNA coupled to an inert carrier molecule, DNA encapsulated in chitin or chitosan, and DNA formulated with an adjuvant. In this context it is noted that practically all considerations pertaining to the use of adjuvants in traditional vaccine formulation apply for the formulation of DNA vaccines. Hence, all disclosures herein which relate to use of adjuvants in the context of polypeptide based vaccines apply *mutatis mutandis* to their use in nucleic acid vaccination technology.

As for routes of administration and administration schemes of polypeptide based vaccines which have been detailed above, these are also applicable for the nucleic acid vaccines of the invention and all discussions above pertaining to routes of administration and administration schemes for polypeptides apply *mutatis mutandis* to nucleic acids. To this should be added that nucleic acid vaccines can suitably be administered intraveneously and intraarterially. Furthermore, it is well-known in the art that nucleic acid vaccines can be administered by use of a so-called gene gun, and hence also this and equivalent modes of administration are regarded as part of the present invention. Finally, also the use of a VLN in the

administration of nucleic acids has been reported to yield good results, and therefore this particular mode of administration is particularly preferred.

Furthermore, the nucleic acid(s) used as an immunization agent can contain regions encoding the 1st, 2nd and/or 3rd moieties, e.g. in the form of the immunomodulating substances described above such as the cytokines discussed as useful adjuvants. A preferred version of this embodiment encompasses having the coding region for the analogue and the coding region for the immunomodulator in different reading frames or at least under the control of different promoters. Thereby it is avoided that the analogue or epitope is produced as a fusion partner to the immunomodulator. Alternatively, two distinct nucleotide fragments can be used, but this is less preferred because of the advantage of ensured co-expression when having both coding regions included in the same molecule.

Accordingly, the invention also relates to a composition for inducing production of antibodies against IL-5, the composition comprising

- a nucleic acid fragment or a vector of the invention (cf. the discussion of vectors below), and
- a pharmaceutically and immunologically acceptable vehicle and/or carrier and/or adjuvant as discussed above.

Under normal circumstances, the IL-5 variant-encoding nucleic acid is introduced in the form of a vector wherein expression is under control of a viral promoter. For more detailed discussions of vectors and DNA fragments according to the invention, cf. the discussion below. Also, detailed disclosures relating to the formulation and use of nucleic acid vaccines are available, cf. Donnelly JJ *et al*, 1997, Annu. Rev. Immunol. **15**: 617-648 and Donnelly JJ *et al.*, 1997, Life Sciences **60**: 163-172. Both of these references are incorporated by reference herein.

Live vaccines

A third alternative for effecting presentation of modified IL-5 to the immune system is the use of live vaccine technology. In live vaccination, presentation to the immune system is effected by administering, to the animal, a non-pathogenic microorganism which has been transformed with a nucleic acid fragment encoding a modified IL-5 or with a vector incorporating such a nucleic acid fragment. The non-pathogenic microorganism can be any suitable attenuated bacterial strain (attenuated by means of passaging or by means of removal of pathogenic expression products by recombinant DNA technology), e.g. *Mycobacterium bovis* BCG., non-pathogenic *Streptococcus* spp., *E. coli*, *Salmonella* spp., *Vibrio cholerae*, *Shigella*, etc. Reviews dealing with preparation of state-of-the-art live vaccines can e.g. be found in Saliou P, 1995, Rev. Prat. **45**: 1492-1496 and Walker PD, 1992, Vaccine **10**: 977-990, both incorporated by reference herein. For details about the nucleic acid fragments and vectors used in such live vaccines, cf. the discussion below.

As an alternative to bacterial live vaccines, the nucleic acid fragment of the invention discussed below can be incorporated in a non-virulent viral vaccine vector such as a vaccinia strain or any other suitable pox virus.

Normally, the non-pathogenic microorganism or virus is administered only once to the animal, but in certain cases it may be necessary to administer the microorganism more than once in a lifetime in order to maintain protective immunity. It is even contemplated that immunization schemes as those detailed above for polypeptide vaccination will be useful when using live or virus vaccines.

Alternatively, live or virus vaccination is combined with previous or subsequent polypeptide and/or nucleic acid vaccination. For instance, it is possible to effect primary

immunization with a live or virus vaccine followed by subsequent booster immunizations using the polypeptide or nucleic acid approach.

The microorganism or virus can be transformed with nucleic acid(s) containing regions encoding the 1st, 2nd and/or 3rd moieties, e.g. in the form of the immunomodulating substances described above such as the cytokines discussed as useful adjuvants. A preferred version of this embodiment encompasses having the coding region for the analogue and the coding region for the immunomodulator in different reading frames or at least under the control of different promoters. Thereby it is avoided that the analogue or epitopes are produced as fusion partners to the immunomodulator. Alternatively, two distinct nucleotide fragments can be used as transforming agents. Of course, having the 1st and/or 2nd and/or 3rd moieties in the same reading frame can provide as an expression product, an analogue of the invention, and such an embodiment is especially preferred according to the present invention.

Use of the method of the invention in disease treatment

As will be appreciated from the discussions above, the provision of the method of the invention allows for control of diseases characterized by eosinophilia. In this context, asthma is the key target for the inventive method but also other chronic allergic conditions such as multiple allergy and allergic rhinitis are feasible targets for treatment/amelioration. Hence, an important embodiment of the method of the invention for down-regulating IL-5 activity comprises treating and/or preventing and/or ameliorating asthma or other chronic allergic conditions characterized by eosinophilia, the method comprising down-regulating IL-5 activity according to the method of the invention to such an extent that the number of eosinophil cells is significantly reduced.

In the present context such a significant reduction in eosinophil cell numbers is at least 20% compared to the eosinophil number prior to treatment, but higher percentages are contemplated, such as at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% and even at least 90%. The reduction may be systemic or, more often, locally in e.g. the lungs.

Eosinophil cell numbers are determined by methods known in the art, typically using microscopy of a suitable sample (such as a BAL fluid) and counting the number of eosinophil cells manually under microscope. Alternatively, eosinophil numbers can be counted using flow cytometric methods or any other convenient method of cytometry capable of distinguishing eosinophils.

Peptides, polypeptides, and compositions of the invention

As will be apparent from the above, the present invention is based on the concept of immunising individuals against the IL-5 antigen in order to indirectly obtain a reduction in eosinophil cell numbers. The preferred way of obtaining such an immunization is to use modified versions of IL-5, thereby providing molecules which have not previously been disclosed in the art.

It is believed that the modified IL-5 molecules discussed herein are inventive in their own right, and therefore an important part of the invention pertains to an IL-5 analogue which is derived from an animal IL-5 wherein is introduced a modification which has as a result that immunization of the animal with the analogue induces production of antibodies cross-reacting with the unmodified IL-5 polypeptide. Preferably, the nature of the modification conforms with the types of modifications described above when discussing various embodiments of the method of the invention when using modified IL-5. Hence, any disclosure presented herein pertaining to modified IL-5 molecules are

relevant for the purpose of describing the IL-5 analogues of the invention, and any such disclosures apply *mutatis mutandis* to the description of these analogues.

It should be noted that preferred modified IL-5 molecules comprise modifications which results in a polypeptide having a sequence identity of at least 70% with IL-5 or with a subsequence thereof of at least 10 amino acids in length. Higher sequence identities are preferred, e.g. at least 75% or even at least 80% or 85%. The sequence identity for proteins and nucleic acids can be calculated as $(N_{ref} - N_{dif}) \cdot 100/N_{ref}$, wherein N_{dif} is the total number of non-identical residues in the two sequences when aligned and wherein N_{ref} is the number of residues in one of the sequences. Hence, the DNA sequence AGTCAGTC will have a sequence identity of 75% with the sequence AATCAATC (N_{dif}=2 and N_{ref}=8).

The invention also pertains to compositions useful in exercising the method of the invention. Hence, the invention also relates to an immunogenic composition comprising an immunogenically effective amount of an IL-5 polypeptide which is a self-protein in an animal, said IL-5 polypeptide being formulated together with an immunologically acceptable adjuvant so as to break the animal's autotolerance towards the IL-5 polypeptide, the composition further comprising a pharmaceutically and immunologically acceptable vehicle and/or carrier. In other words, this part of the invention pertains to the formulations of naturally occurring IL-5 polypeptides which have been described in connection with embodiments of the method of the invention.

The invention also relates to an immunogenic composition comprising an immunologically effective amount of an IL-5 analogue defined above, said composition further comprising a pharmaceutically and immunologically acceptable diluent and/or vehicle and/or carrier and/or excipient and optionally an

adjuvant. In other words, this part of the invention concerns formulations of modified IL-5, essentially as described hereinabove. The choice of adjuvants, carriers, and vehicles is accordingly in line with what has been discussed above when referring to formulation of modified and unmodified IL-5 for use in the inventive method for the down-regulation of IL-5.

The polypeptides are prepared according to methods well-known in the art. Longer polypeptides are normally prepared by means of recombinant gene technology including introduction of a nucleic acid sequence encoding the IL-5 analogue into a suitable vector, transformation of a suitable host cell with the vector, expression of the nucleic acid sequence, recovery of the expression product from the host cells or their culture supernatant, and subsequent purification and optional further modification, e.g. refolding or derivatization.

Shorter peptides are preferably prepared by means of the well-known techniques of solid- or liquid-phase peptide synthesis. However, recent advances in this technology has rendered possible the production of full-length polypeptides and proteins by these means, and therefore it is also within the scope of the present invention to prepare the long constructs by synthetic means.

Nucleic acid fragments and vectors of the invention

It will be appreciated from the above disclosure that modified IL-5 polypeptides can be prepared by means of recombinant gene technology but also by means of chemical synthesis or semisynthesis; the latter two options are especially relevant when the modification consists in coupling to protein carriers (such as KLH, diphtheria toxoid, tetanus toxoid, and BSA) and non-proteinaceous molecules such as carbohydrate polymers and of course also when the modification comprises addition of side

chains or side groups to an IL-5 polypeptide-derived peptide chain.

For the purpose of recombinant gene technology, and of course also for the purpose of nucleic acid immunization, nucleic acid fragments encoding modified IL-5 are important chemical products. Hence, an important part of the invention pertains to a nucleic acid fragment which encodes an IL-5 analogue, *i.e.* an IL-5 derived polypeptide which either comprises the natural IL-5 sequence to which has been added or inserted a fusion partner or, preferably an IL-5 derived polypeptide wherein has been introduced a foreign T-cell epitope by means of insertion and/or addition, preferably by means of substitution and/or deletion. The nucleic acid fragments of the invention are either DNA or RNA fragments.

The nucleic acid fragments of the invention will normally be inserted in suitable vectors to form cloning or expression vectors carrying the nucleic acid fragments of the invention; such novel vectors are also part of the invention. Details concerning the construction of these vectors of the invention will be discussed in context of transformed cells and microorganisms below. The vectors can, depending on purpose and type of application, be in the form of plasmids, phages, cosmids, mini-chromosomes, or virus, but also naked DNA which is only expressed transiently in certain cells is an important vector. Preferred cloning and expression vectors of the invention are capable of autonomous replication, thereby enabling high copy-numbers for the purposes of high-level expression or high-level replication for subsequent cloning.

The general outline of a vector of the invention comprises the following features in the 5'-3' direction and in operable linkage: a promoter for driving expression of the nucleic acid fragment of the invention, optionally a nucleic acid sequence encoding a leader peptide enabling secretion (to the

extracellular phase or, where applicable, into the periplasma) of or integration into the membrane of the polypeptide fragment, the nucleic acid fragment of the invention, and optionally a nucleic acid sequence encoding a terminator. When operating with expression vectors in producer strains or cell-lines it is for the purposes of genetic stability of the transformed cell preferred that the vector when introduced into a host cell is integrated in the host cell genome. In contrast, when working with vectors to be used for effecting *in vivo* expression in an animal (*i.e.* when using the vector in DNA vaccination) it is for security reasons preferred that the vector is not incapable of being integrated in the host cell genome; typically, naked DNA or non-integrating viral vectors are used, the choices of which are well-known to the person skilled in the art.

The vectors of the invention are used to transform host cells to produce the modified IL-5 polypeptide of the invention. Such transformed cells, which are also part of the invention, can be cultured cells or cell lines used for propagation of the nucleic acid fragments and vectors of the invention, or used for recombinant production of the modified IL-5 polypeptides of the invention. Alternatively, the transformed cells can be suitable live vaccine strains wherein the nucleic acid fragment (one single or multiple copies) have been inserted so as to effect secretion or integration into the bacterial membrane or cell-wall of the modified IL-5.

Preferred transformed cells of the invention are microorganisms such as bacteria (such as the species *Escherichia* [e.g. *E.coli*], *Bacillus* [e.g. *Bacillus subtilis*], *Salmonella*, or *Mycobacterium* [preferably non-pathogenic, e.g. *M. bovis* BCG]), yeasts (such as *Saccharomyces cerevisiae*), and protozoans. Alternatively, the transformed cells are derived from a multicellular organism such as a fungus, an insect cell, a plant cell, or a mammalian cell. Most preferred are cells derived from a human being, cf. the discussion of cell lines and vectors below. Recent results have

shown great promise in the use of a commercially available *Drosophila melanogaster* cell line (the Schneider 2 (S_2)cell line and vector system available from Invitrogen) for the recombinant production of IL-5 analogues of the invention, and therefore this expression system is particularly preferred.

For the purposes of cloning and/or optimized expression it is preferred that the transformed cell is capable of replicating the nucleic acid fragment of the invention. Cells expressing the nucleic fragment are preferred useful embodiments of the invention; they can be used for small-scale or large-scale preparation of the modified IL-5 or, in the case of non-pathogenic bacteria, as vaccine constituents in a live vaccine.

When producing the modified IL-5 of the invention by means of transformed cells, it is convenient, although far from essential, that the expression product is either exported out into the culture medium or carried on the surface of the transformed cell.

When an effective producer cell has been identified it is preferred, on the basis thereof, to establish a stable cell line which carries the vector of the invention and which expresses the nucleic acid fragment encoding the modified IL-5. Preferably, this stable cell line secretes or carries the IL-5 analogue of the invention, thereby facilitating purification thereof.

In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with the hosts. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* is typically transformed using pBR322, a plasmid derived from an *E. coli* species (see, e.g., Bolivar et al., 1977). The pBR322 plasmid contains genes

for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells. The pBR plasmid, or other microbial plasmid or phage must also contain, or be modified to contain, promoters which can be used by the prokaryotic microorganism for expression.

Those promoters most commonly used in prokaryotic recombinant DNA construction include the B-lactamase (penicillinase) and lactose promoter systems (Chang et al., 1978; Itakura et al., 1977; Goeddel et al., 1979) and a tryptophan (trp) promoter system (Goeddel et al., 1979; EP-A-0 036 776). While these are the most commonly used, other microbial promoters have been discovered and utilized, and details concerning their nucleotide sequences have been published, enabling a skilled worker to ligate them functionally with plasmid vectors (Siebwenlist et al., 1980). Certain genes from prokaryotes may be expressed efficiently in *E. coli* from their own promoter sequences, precluding the need for addition of another promoter by artificial means.

In addition to prokaryotes, eukaryotic microbes, such as yeast cultures may also be used, and here the promoter should be capable of driving expression. *Saccharomyces cerevisiase*, or common baker's yeast is the most commonly used among eukaryotic microorganisms, although a number of other strains are commonly available. For expression in *Saccharomyces*, the plasmid YRp7, for example, is commonly used (Stinchcomb et al., 1979; Kingsman et al., 1979; Tschemper et al., 1980). This plasmid already contains the trpl gene which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan for example ATCC No. 44076 or PEP4-1 (Jones, 1977). The presence of the trpl lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

Suitable promoting sequences in yeast vectors include the promoters for 3-phosphoglycerate kinase (Hitzman et al., 1980) or other glycolytic enzymes (Hess et al., 1968; Holland et al., 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. In constructing suitable expression plasmids, the termination sequences associated with these genes are also ligated into the expression vector 3' of the sequence desired to be expressed to provide polyadenylation of the mRNA and termination.

Other promoters, which have the additional advantage of transcription controlled by growth conditions are the promoter region for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, and the aforementioned glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Any plasmid vector containing a yeast-compatible promoter, origin of replication and termination sequences is suitable.

In addition to microorganisms, cultures of cells derived from multicellular organisms may also be used as hosts. In principle, any such cell culture is workable, whether from vertebrate or invertebrate culture. However, interest has been greatest in vertebrate cells, and propagation of vertebrate in culture (tissue culture) has become a routine procedure in recent years (Tissue Culture, 1973). Examples of such useful host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, and W138, BHK, COS-7 293, *Spodoptera frugiperda* (SF) cells (commercially available as complete expression systems from *i.a.* Protein Sciences, 1000 Research Parkway, Meriden, CT 06450, U.S.A. and from Invitrogen), and MDCK cell lines. In the present invention, an especially preferred cell line is S_2 available

from Invitrogen, PO Box 2312, 9704 CH Groningen, The Netherlands.

Expression vectors for such cells ordinarily include (if necessary) an origin of replication, a promoter located in front of the gene to be expressed, along with any necessary ribosome binding sites, RNA splice sites, polyadenylation site, and transcriptional terminator sequences.

For use in mammalian cells, the control functions on the expression vectors are often provided by viral material. For example, commonly used promoters are derived from polyoma, Adenovirus 2, and most frequently Simian Virus 40 (SV40). The early and late promoters of SV40 virus are particularly useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication (Fiers et al., 1978). Smaller or larger SV40 fragments may also be used, provided there is included the approximately 250 bp sequence extending from the *Hind*III site toward the BglI site located in the viral origin of replication. Further, it is also possible, and often desirable, to utilize promoter or control sequences normally associated with the desired gene sequence, provided such control sequences are compatible with the host cell systems.

An origin of replication may be provided either by construction of the vector to include an exogenous origin, such as may be derived from SV40 or other viral (e.g., Polyoma, Adeno, VSV, BPV) or may be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter is often sufficient.

Identification of useful IL-5 analogues

It will be clear to the skilled person that not all variants or modifications of native IL-5 will have the ability to elicit

antibodies in an animal which are cross-reactive with the native form. It is, however, not difficult to set up an effective standard screen for modified IL-5 molecules which fulfill the minimum requirements for immunological reactivity discussed herein. Hence, another part of the invention concerns a method for the identification of a modified IL-5 polypeptide which is capable of inducing antibodies against unmodified IL-5 in an animal species where the unmodified IL-5 polypeptide is a self-protein, the method comprising

- preparing, by means of peptide synthesis or by molecular biological means, a set of mutually distinct modified IL-5 polypeptides wherein amino acids have been added to, inserted in, deleted from, or substituted into the amino acid sequence of an IL-5 polypeptide of the animal species thereby giving rise to amino acid sequences in the set which comprise T-cell epitopes which are and foreign to the animal species,
- testing members of the set for their ability to induce production of antibodies by the animal species against the unmodified IL-5, and
- selecting and isolating the member(s) of the set which significantly induces antibody production against unmodified IL-5 in the animal species.

In this context, the "set of mutually distinct modified IL-5 polypeptides" is a collection of non-identical modified IL-5 polypeptides which have e.g. been selected on the basis of the criteria discussed above (e.g. in combination with studies of circular dichroism, NMR spectra, and/or X-ray diffraction patterns). The set may consist of only a few members but it is contemplated that the set may contain several hundred members.

The test of members of the set can ultimately be performed *in vivo*, but a number of *in vitro* tests can be applied which narrow

down the number of modified molecules which will serve the purpose of the invention.

Since the goal of introducing the foreign T-cell epitopes is to support the B-cell response by T-cell help, a prerequisite is that T-cell proliferation is induced by the modified IL-5. T-cell proliferation can be tested by standardized proliferation assays *in vitro*. In short, a sample enriched for T-cells is obtained from a subject and subsequently kept in culture. The cultured T-cells are contacted with APCs of the subject which have previously taken up the modified molecule and processed it to present its T-cell epitopes. The proliferation of T-cells is monitored and compared to a suitable control (e.g. T-cells in culture contacted with APCs which have processed intact, native IL-5). Alternatively, proliferation can be measured by determining the concentration of relevant cytokines released by the T-cells in response to their recognition of foreign T-cells.

Having rendered highly probable that at least one modified IL-5 of the set is capable of inducing antibody production against IL-5, it is possible to prepare an immunogenic composition comprising at least one modified IL-5 polypeptide which is capable of inducing antibodies against unmodified IL-5 in an animal species where the unmodified IL-5 polypeptide is a self-protein, the method comprising admixing the member(s) of the set which significantly induces production of antibodies in the animal species which are reactive with IL-5 with a pharmaceutically and immunologically acceptable carrier and/or vehicle and/or diluent and/or excipient, optionally in combination with at least one pharmaceutically and immunologically acceptable adjuvant.

The above aspects of the invention are conveniently carried out by initially preparing a number of mutually distinct nucleic acid sequences or vectors of the invention, inserting these into appropriate expression vectors, transforming suitable host cells

with the vectors, and expressing the nucleic acid sequences of the invention. These steps can be followed by isolation of the expression products. It is preferred that the nucleic acid sequences and/or vectors are prepared by methods comprising exercise of a molecular amplification technique such as PCR or by means of nucleic acid synthesis.

PREAMBLE TO EXAMPLES

Vaccine design

The exemplary candidates for an IL-5 autovaccine are constructed according to the AutoVac™ concept (described in detail in WO 95/05849) by substitution with known promiscuous T cell epitopes into the human IL-5 wild type protein. The substitutions are peptide substitutions, where the inserted peptide may be of the same or different length than the deleted peptide in the wild-type sequence.

For initial proof of concept by *in vivo* testing and screening, it is planned to prepare the constructs in the murine IL-5 sequence. By way of example, the tetanus toxoid epitopes P2 (SEQ ID NO: 23) and P30 (SEQ ID NO: 24) are used as substituting peptides, but any other suitable peptide containing or constituting a promiscuous T_H epitope could, according to the present invention, be used.

It should be emphasized that the size of the molecule (115 res.) compared to the size of the substitutions (15 or 21 residues for P2 and P30, respectively) strongly limits the possible sites of structural non-destructive inserts. As the disulfide bridges are important, but not imperative, for the dimerisation, some variants are made in pairs +/- elimination of the cysteines.

In the construction of the candidate molecules, two basic parameters have been considered. First, it is attempted to conserve a maximum fraction of the three dimensional structure of the wild type hIL-5, thereby conserving the native B-cell epitope repertoire. This is supported by Dickason et al., (1994) who demonstrated that IL-5 B-cell epitopes known to be neutralising are conformational. Conservation of the tertiary structure is sought achieved by introducing the modifications at structurally "neutral" sites, such as loops or separate segments. The fact that the N-terminal helix "A" together with the helices "B" and "C" are able to fold into a quaternary structure with a second molecule, indicates that these 3 helices constitute a stable folding-scaffold.

Second, the biological activity in relation to the vaccine concept has been considered. In general, an inactive construct is preferable with a view to reducing putative toxic effects of the molecules and in general for evaluating the immune response. On the other hand, the optimum neutralising antibodies should theoretically exhibit specificity for the part of IL-5 that interacts with the IL-5R. This is most likely achieved by immunising with an active variant. Finally, it is not impossible that the biological effect of IL-5 on the immune system might act as an enhancer on the immune response, thus improving the overall effect. Based on Applicant's previous experiences with other molecules, however, the majority of "theoretically possible active" constructs is expected to have low or no activity.

Therefore, all variants suggested are potentially active but can, if desirable, with relative ease be rendered inactive by hindering the formation of the active dimer or by alterations in the areas of the "A"- and "D"-helices that are involved in the receptor binding/activation.

In summary, the above considerations of structure conservation and biological activity defines the target areas as any one of loops 1-3 as well as the C-terminal flexible area.

Loop 3 is selected as the primary target area since it is structurally separated from the assumed tri-helical folding scaffold. As it is furthermore possible to produce a biologically active monomer, by elongation of loop 3 (Dickason, 1996), this area holds the possibilities for generating all types of variants: monomer/dimer and active/inactivated.

"Loop 1" is a second area containing a non-helical stretch of a suitable length for substitutions. Variants from this region would theoretically be active only if capable of dimerising, but since the length of the wild-type loop makes it rather flexible it is reasonable to expect a correct folding of the protein after substitution.

Variants containing substitutions in the "loop 2" area will also only be active as dimers. The area that can be substituted is short compared to the inserts and has a central position in the assumed folding scaffold, two characteristics of loop 2 which might be of hindrance to the correct folding of the protein after substitution. On the other hand, loop 2 is situated opposite to the area interacting with the IL-5R, resulting in an expected optimum presentation of the wild-type neutralising epitopes if the modified protein is correctly folded.

Finally, inserts in the C-terminal flexible region following "helix D" are proposed. From a protein structure point of view this concept appears fairly safe, but it is likely that modifications in this region will affect both dimerisation and biological activity (if the modified protein is dimerised) since the C-terminal is located in the area of both receptor binding and in the dimer interface.

The amino acid sequence of 10 variants constructed according to the above considerations are set forth as SEQ ID NOs: 2-11 and 13-22.

It should be noted, that all inserts except from the ones according to Example 2 are prepared so as to include flanking amino acid residues that are conserved from hIL-5 to mIL-5 in order to promote the process of successful transfer of positive constructs from mice to man.

In the following examples, positions for substitution are indexed according to the murine amino acid residue sequence numbers; the corresponding human positions are given in parentheses.

EXAMPLE 1

Variants of type #1: P2 substituting positions 85-88 (87-90)

The P2 epitope (SEQ ID NO: 23) is substituted into loop 3 while avoiding elimination of Cys84(86). These variants (SEQ ID NOs: 2 and 13) are potentially active as both monomers (due to the elongation of loop 3) and as dimers.

EXAMPLE 2

Variants of type #2: P2 substituting positions 31-41 (33-43)

The P2 epitope (SEQ ID NO: 23) is substituted into loop 1 while avoiding elimination of Cys42(44). These variants (SEQ ID NOs: 3 and 14) are potentially active as dimers only.

EXAMPLE 3

Variants of type #3: P2 substituting positions 57-62 (59-64)

The P2 epitope (SEQ ID NO: 23) is substituted into loop 3. These variants (SEQ ID NOs: 4 and 15) are potentially active as dimers only.

EXAMPLE 4

Variants of type #4: P2 substituting positions 84-89 (86-91)

The P2 epitope (SEQ ID NO: 23) is substituted into loop 3 while *eliminating* Cys84(86). These variants (SEQ ID NOs: 5 and 16) are in principle similar to the variants of type #1 (SEQ ID NOs: 2 and 13), but the generation of monomer products has been facilitated by inhibiting the formation of disulfide bridging and adjusting the length of loop 3.

EXAMPLE 5

Variants of type #5: P2 substituting positions 108-111(110-113)

The P2 epitope (SEQ ID NO: 23) is substituted into the C-terminal area succeeding helix D. These variants (SEQ ID NOs: 6 and 17) are potentially active as a dimer only.

EXAMPLE 6

Variants of type #6: P30 substituting positions 85-88 (87-90)

The P30 epitope (SEQ ID NO: 24) is substituted into loop 3 avoiding elimination of Cys84(86). These variants (SEQ ID NOs: 7

and 18) are potentially active both as monomers (due to the elongation of loop 3) and as dimers.

EXAMPLE 7

Variants of type #7: P30 substituting positions 30-41 (32-43)

The P30 epitope (SEQ ID NO: 24) is substituted into loop 1, avoiding elimination of Cys42(44). These variants (SEQ ID NOs: 8 and 19) are potentially active as dimers only.

EXAMPLE 8

Variants of type #8: P30 substituting positions 57-62 (59-64)

The P30 epitope (SEQ ID NO: 24) is substituted into loop 3. These variants (SEQ ID NOs: 9 and 20) are potentially active as dimers only.

EXAMPLE 9

Variants of type #9: P30 substituting positions 108-111(110-113)

The P30 epitope (SEQ ID NO: 24) is substituted into the C-terminal area succeeding helix D. These variants (SEQ ID NOs: 10 and 21) are potentially active as dimers only.

EXAMPLE 10

Variants of type #10: P2 substituting positions 84-89 (86-91) and P30 substituting positions 110-113

The P2 epitope (SEQ ID NO: 23) is substituted into loop 3 eliminating Cys84(86) and the P30 epitope (SEQ ID NO: 24) is substituted into the C-terminal area succeeding helix-D. These variants (SEQ ID NOs: 11 and 22) are the only ones containing both epitopes and are potentially active monomers.

EXAMPLE 11

Choise of expression system

Recombinant IL-5 has been expressed in a number of different expression systems including yeast, insect cells and CHO cells (Tavernier *et al.*, 1989).

According to the present invention, one suitable expression system is *E. coli*, based on previous studies reporting the use of this host for production hIL-5 (Proudfoot *et al.*, 1990, Graber *et al.*, 1993). The recombinant protein is expressed as inclusion bodies that are converted into the biologically active dimer upon purification and re-folding (e.g. using the generally applicable refolding methods disclosed in US 5,739,281). The speed and simplicity of *E. coli* expression allows immediate initiation of the production of protein when the genetic constructs are ready, thus facilitating rapid generation of material to establish an *in vivo* proof of the IL-5 autovaccine concept.

If for some reason the feasibility is found to be to low (e.g. low yield following re-folding, instability of the products or improved pharmacokinetical parameters related to glycosylation

etc), production in yeast could be considered in a further development of the autovaccine.

Recently, promising results have been obtained using the *Drosophila melanogaster* expression system using S_2 cells (available from Invitrogen) and at present this system is the preferred embodiment for expression of the IL-5 analogues of the invention. Transient expression of 2 of the variants has been observed and it seems that also stable transformation will be accomplished. Until now, glycosylated variants of Type 1 and 5 have been transiently expressed in this system.

EXAMPLE 12

Screening and selection of the modified molecules

Following expression, the recombinant protein is purified and characterised. The characterisation of the autovaccine candidates will include analytical chromatography, iso-electric focussing (IEF), SDS-PAGE, amino acid composition analysis, N-terminal sequence analysis, mass spectrometry, low angle laser light scattering, standard spectroscopy, and Circular Dichroism to an extent that precisely document the relevant parameters defining the intended protein product.

EXAMPLE 13

In vitro screening

The primary *in vitro* screening will be in the form of an enzyme-linked immunosorbent assay (ELISA): A competitive ELISA towards wild-type IL-5 provides an estimate of the presence of relevant B-cell epitopes in the modified IL-5 constructs before introduction thereof into animals.

A conventional ELISA assay can be used to measure titres of auto-antibodies in the serum of vaccinated animals. Antibodies (both mono-specific and monoclonal) towards the human as well as towards the murine IL-5 are commercially available from R&D Systems, 614 McKinley Place NE, Minneapolis, MN 55413, USA.

The biological activity of the product and/or the neutralising capacity of induced auto-antibodies can be tested in a IL-5 bioassay. Previously reported examples of such bioassays are: Assessment of IL-5 induced proliferation of TF1 cells (for human IL-5) and assessment of IL-5 induced proliferation of BCL1 cells or B13 B cells (for murine IL-5) (Callard & Gearing 1994, Dickason *et al.*, 1994).

The effect on airway responsiveness of the autovaccine can also be tested in an *in vitro* assay wherein the trachea from vaccinated mice are removed and placed on a hook in an organ bath. The tension of the trachea after histamine challenge is measured (van Oosterhout *et al.*, 1995).

EXAMPLE 14

In vivo models

For measuring the *in vivo* effect of the autovaccine, well-known animal models for asthma exists. Normally, the animal is sensitised with a compound (allergen/antigen) and after challenge with the aerosolised compound, broncho-constriction (airway conduction) is measured using a body plethysmograph. The eosinophil cell counts in the BAL fluid are also determined.

Several of the studies investigating the effect of anti-IL5 mAb's have been successfully performed in mice. Against use of the murine model speaks the fact the IL-5 acts as a B-cell growth factor, rendering possible interference with the murine

antibody response. However, as shown in a study using IL-5 knock-out mice, the T-cell dependent antibody response against ovalbumin as well as cytotoxic T-cell development appeared normal (Kopf *et al.*, 1996). As the mouse is also the most practical and economical model in comparison to guinea pigs or monkeys, the ovalbumin sensitised Bal/c mice model of asthma/airway hypersensitivity as used by Hamelman et al. (1997) will be used.

If, however, the effect of IL-5 on B-cells in the murine model turns out to be a problem, the use of other suitable animal models known in the art will be applied.

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SEQUENCE LISTING

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 65                  70                  75                  80

Tyr Ile Asp Gly Gln Lys Lys Lys Cys Gly Glu Glu Arg Arg Arg Val
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Ser Lys Phe Ile Gly Ile Thr Glu Leu Val Glu Arg Leu Phe Lys Asn
65                  70                  75                  80

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         35                  40                  45

Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln Gly Gly Thr
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Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp
 65                  70                  75                  80

Gly Gln Lys Lys Lys Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly
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			20					25					30		

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         35                  40                  45

Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Phe Asn Asn Phe Thr Val
     50                  55                  60

Ser Phe Trp Leu Arg Val Pro Lys Val Ser Ala Ser His Leu Glu Val
 65                  70                  75                  80

Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Gly
                 85                  90                  95

Gln Lys Lys Lys Cys Gly Glu Glu Arg Arg Arg Val Asn Gln Phe Leu
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Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp
65                  70                  75                  80

Gly Gln Lys Lys Lys Cys Gly Glu Glu Arg Arg Arg Val Asn Gln Phe
                85                  90                  95

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<223> Description of Artificial Sequence:Human IL-5
modified by substitution with tetanus toxoid P2
and P30 epitopes

<220>
<221> MUTAGEN
<222> (86)..(100)
<223> Tetanus toxoid P2 epitope (SEQ ID NO: 23)

<220>
<221> MUTAGEN
<222> (119)..(139)

<223> Tetanus toxoid P30 epitope (SEQ ID NO: 24)

<220>
<221> SIMILAR
<222> (1)..(85)
<223> Identical to residues 1-85 in SEQ ID NO: 1

<220>
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<222> (101)..(118)
<223> Identical to residues 92-109 in SEQ ID NO: 1

<220>
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<222> (140)..(141)
<223> Identical to residues 114-115 in SEQ ID NO: 1

<400> 11

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Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
  1               5                  10                  15

Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Arg
             20                  25                  30

Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
         35                  40                  45

Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln Gly Gly Thr
     50                  55                  60

Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp
 65                  70                  75                  80

Gly Gln Lys Lys Lys Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly
                 85                  90                  95

Ile Thr Glu Leu Arg Val Asn Gln Phe Leu Asp Tyr Leu Gln Glu Phe
            100                 105                 110

Leu Gly Val Met Asn Thr Phe Asn Asn Phe Thr Val Ser Phe Trp Leu
        115                 120                 125

Arg Val Pro Lys Val Ser Ala Ser His Leu Glu Glu Ser
    130                 135                 140
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<210> 12
<211> 113
<212> PRT
<213> Mus musculus

<220>
<221> DISULFID
<222> (42)

<223> Interchain disulphide bond to Cys-84 in SEQ ID NO:12

<220>
<221> DISULFID
<222> (84)
<223> Interchain disulphide bond to Cys-42 in SEQ ID NO:12

<400> 12

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Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
 1               5                  10                  15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
            20                  25                  30

Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
        35                  40                  45

Gly Leu Asp Ile Leu Lys Asn Gln Thr Val Arg Gly Gly Thr Val Glu
    50                  55                  60

Met Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
65                  70                  75                  80

Lys Glu Lys Cys Gly Glu Glu Arg Arg Arg Thr Arg Gln Phe Leu Asp
                85                  90                  95

Tyr Leu Gln Glu Phe Leu Gly Val Met Ser Thr Glu Trp Ala Met Glu
            100                 105                 110

Gly
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<210> 13
<211> 124
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Murine IL-5 modified by substitution with tetanus toxoid P2 epitope

<220>
<221> MUTAGEN
<222> (85)..(99)
<223> Tetanus toxoid P2 epitope (SEQ ID NO: 23)

<220>
<221> SIMILAR
<222> (1)..(84)
<223> Identical to residues 1-84 in SEQ ID NO: 12

<220>
<221> SIMILAR
<222> (100)..(124)
<223> Identical to residues 89-113 in SEQ ID NO: 12

<400> 13
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1 5 10 15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
20 25 30

Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
35 40 45

Gly Leu Asp Ile Leu Lys Asn Gln Thr Val Arg Gly Gly Thr Val Glu
50 55 60

Met Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
65 70 75 80

Lys Glu Lys Cys Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile
85 90 95

Thr Glu Leu Arg Arg Thr Arg Gln Phe Leu Asp Tyr Leu Gln Glu Phe
100 105 110

Leu Gly Val Met Ser Thr Glu Trp Ala Met Glu Gly
115 120

<210> 14
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Murine IL-5 modified by substitution with tetanus toxoid P2 epitope

<220>
<221> MUTAGEN
<222> (30)..(44)
<223> Tetanus toxoid P2 epitope (SEQ ID NO: 23)

<220>
<221> SIMILAR
<222> (1)..(29)
<223> Identical to residues 1-29 in SEQ ID NO: 12

<220>
<221> SIMILAR
<222> (45)..(116)

<223> Identical to residues 42-113 in SEQ ID NO: 12

<400> 14

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Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
 1               5                  10                  15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Gln Tyr Ile
            20                  25                  30

Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr Glu Leu Cys Ile Gly Glu
        35                  40                  45

Ile Phe Gln Gly Leu Asp Ile Leu Lys Asn Gln Thr Val Arg Gly Gly
    50                  55                  60

Thr Val Glu Met Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile
65                  70                  75                  80

Asp Arg Gln Lys Glu Lys Cys Gly Glu Glu Arg Arg Arg Thr Arg Gln
                85                  90                  95

Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Ser Thr Glu Trp
            100                 105                 110

Ala Met Glu Gly
        115
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<210> 15
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Murine IL-5 modified by substitution with tetanus toxoid P2 epitope

<220>
<221> MUTAGEN
<222> (57)..(71)
<223> Tetanus toxoid P2 epitope (SEQ ID NO: 23)

<220>
<221> SIMILAR
<222> (1)..(56)
<223> Identical to residues 1-56 in SEQ ID NO: 12

<220>
<221> SIMILAR
<222> (72)..(122)
<223> Identical to residues 63-113 in SEQ ID NO: 12

<400> 15

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Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
  1               5                  10                  15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
             20                  25                  30

Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
         35                  40                  45

Gly Leu Asp Ile Leu Lys Asn Gln Gln Tyr Ile Lys Ala Asn Ser Lys
     50                  55                  60

Phe Ile Gly Ile Thr Glu Leu Val Glu Met Leu Phe Gln Asn Leu Ser
 65                  70                  75                  80

Leu Ile Lys Lys Tyr Ile Asp Arg Gln Lys Glu Lys Cys Gly Glu Glu
                 85                  90                  95

Arg Arg Arg Thr Arg Gln Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly
            100                 105                 110

Val Met Ser Thr Glu Trp Ala Met Glu Gly
        115                 120
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<210> 16
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Murine IL-5 modified by substitution with tetanus toxoid P2 epitope

<220>
<221> MUTAGEN
<222> (84)..(98)
<223> Tetanus toxoid P2 epitope (SEQ ID NO: 23)

<220>
<221> SIMILAR
<222> (1)..(83)
<223> Identical to residues 1-83 in SEQ ID NO: 12

<220>
<221> SIMILAR
<222> (99)..(122)
<223> Identical to residues 90-113 in SEQ ID NO: 12

<400> 16

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Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
  1               5                  10                  15
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Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
20 25 30

Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
35 40 45

Gly Leu Asp Ile Leu Lys Asn Gln Thr Val Arg Gly Gly Thr Val Glu
50 55 60

Met Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
65 70 75 80

Lys Glu Lys Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr
85 90 95

Glu Leu Arg Thr Arg Gln Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly
100 105 110

Val Met Ser Thr Glu Trp Ala Met Glu Gly
115 120

<210> 17
<211> 124
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Murine IL-5 modified by substitution with tetanus toxoid P2 epitope

<220>
<221> MUTAGEN
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<223> Tetanus toxoid P2 epitope (SEQ ID NO: 23)

<220>
<221> SIMILAR
<222> (1)..(107)
<223> Identical to residues 1-107 in SEQ ID NO: 12

<220>
<221> SIMILAR
<222> (123)..(124)
<223> Identical to residues 112-113 in SEQ ID NO: 12

<400> 17
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
1 5 10 15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
20 25 30

Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
35 40 45

Gly Leu Asp Ile Leu Lys Asn Gln Thr Val Arg Gly Gly Thr Val Glu
50 55 60

Met Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
65 70 75 80

Lys Glu Lys Cys Gly Glu Glu Arg Arg Arg Thr Arg Gln Phe Leu Asp
85 90 95

Tyr Leu Gln Glu Phe Leu Gly Val Met Ser Thr Gln Tyr Ile Lys Ala
100 105 110

Asn Ser Lys Phe Ile Gly Ile Thr Glu Leu Glu Gly
115 120

<210> 18
<211> 130
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Murine IL-5 modified by substitution with tetanus toxoid P30 epitope

<220>
<221> MUTAGEN
<222> (85)..(105)
<223> Tetanus toxoid P2 epitope (SEQ ID NO: 24)

<220>
<221> SIMILAR
<222> (1)..(84)
<223> Identical to residues 1-84 in SEQ ID NO: 12

<220>
<221> SIMILAR
<222> (106)..(130)
<223> Identical to residues 89-113 in SEQ ID NO: 12

<400> 18
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
1 5 10 15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
20 25 30

Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
35 40 45

Gly Leu Asp Ile Leu Lys Asn Gln Thr Val Arg Gly Gly Thr Val Glu
50 55 60

Met Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
65 70 75 80

Lys Glu Lys Cys Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val
85 90 95

Pro Lys Val Ser Ala Ser His Leu Glu Arg Arg Thr Arg Gln Phe Leu
100 105 110

Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Ser Thr Glu Trp Ala Met
115 120 125

Glu Gly
130

<210> 19
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Murine IL-5 modified by substitution with tetanus toxoid P30 epitope

<220>
<221> MUTAGEN
<222> (30)..(50)
<223> Tetanus toxoid P30 epitope (SEQ ID NO: 24)

<220>
<221> SIMILAR
<222> (1)..(29)
<223> Identical to residues 1-29 in SEQ ID NO: 12

<220>
<221> SIMILAR
<222> (51)..(122)
<223> Identical to residues 42-113 in SEQ ID NO: 12

<400> 19
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1 5 10 15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Phe Asn Asn
20 25 30

Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys Val Ser Ala Ser His
35 40 45

```
Leu Glu Cys Ile Gly Glu Ile Phe Gln Gly Leu Asp Ile Leu Lys Asn
     50                  55                  60

Gln Thr Val Arg Gly Gly Thr Val Glu Met Leu Phe Gln Asn Leu Ser
 65                  70                  75                  80

Leu Ile Lys Lys Tyr Ile Asp Arg Gln Lys Glu Lys Cys Gly Glu Glu
                 85                  90                  95

Arg Arg Arg Thr Arg Gln Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly
            100                 105                 110

Val Met Ser Thr Glu Trp Ala Met Glu Gly
        115                 120
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<210> 20
<211> 128
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Murine IL-5 modified by substitution with tetanus toxoid P30 epitope

<220>
<221> MUTAGEN
<222> (57)..(77)
<223> Tetanus toxoid P30 epitope (SEQ ID NO: 24)

<220>
<221> SIMILAR
<222> (1)..(56)
<223> Identical to residues 1-56 in SEQ ID NO: 12

<220>
<221> SIMILAR
<222> (78)..(128)
<223> Identical to residues 63-113 in SEQ ID NO: 12

<400> 20

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  1               5                  10                  15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
             20                  25                  30

Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
         35                  40                  45

Gly Leu Asp Ile Leu Lys Asn Gln Phe Asn Asn Phe Thr Val Ser Phe
     50                  55                  60
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Trp Leu Arg Val Pro Lys Val Ser Ala Ser His Leu Glu Val Glu Met
65 70 75 80

Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln Lys
85 90 95

Glu Lys Cys Gly Glu Glu Arg Arg Arg Thr Arg Gln Phe Leu Asp Tyr
100 105 110

Leu Gln Glu Phe Leu Gly Val Met Ser Thr Glu Trp Ala Met Glu Gly
115 120 125

<210> 21
<211> 130
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Murine IL-5 modified by substitution with tetanus toxoid P30 epitope

<220>
<221> MUTAGEN
<222> (108)..(128)
<223> Tetanus toxoid P30 epitope (SEQ ID NO: 24)

<220>
<221> SIMILAR
<222> (1)..(107)
<223> Identical to residues 1-107 in SEQ ID NO: 12

<220>
<221> SIMILAR
<222> (129)..(130)
<223> Identical to residues 112-113 in SEQ ID NO: 12

<400> 21
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1 5 10 15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
20 25 30

Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
35 40 45

Gly Leu Asp Ile Leu Lys Asn Gln Thr Val Arg Gly Gly Thr Val Glu
50 55 60

Met Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
65 70 75 80

Lys Glu Lys Cys Gly Glu Glu Arg Arg Arg Thr Arg Gln Phe Leu Asp
85 90 95

Tyr Leu Gln Glu Phe Leu Gly Val Met Ser Thr Phe Asn Asn Phe Thr
100 105 110

Val Ser Phe Trp Leu Arg Val Pro Lys Val Ser Ala Ser His Leu Glu
115 120 125

Glu Gly
130

<210> 22
<211> 139
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Murine IL-5 modified by substitution with tetanus toxoid P2 and P30 epitopes

<220>
<221> MUTAGEN
<222> (84)..(98)
<223> Tetanus toxoid P2 epitope (SEQ ID NO: 23)

<220>
<221> MUTAGEN
<222> (117)..(137)
<223> Tetanus toxoid P30 epitope (SEQ ID NO: 24)

<220>
<221> SIMILAR
<222> (1)..(83)
<223> Identical to residues 1-83 in SEQ ID NO: 12

<220>
<221> SIMILAR
<222> (99)..(116)
<223> Identical to residues 90-107 in SEQ ID NO: 12

<220>
<221> SIMILAR
<222> (138)..(139)
<223> Identical to residues 112-113 in SEQ ID NO: 12

<400> 22
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
1 5 10 15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
20 25 30

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Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
         35                  40                  45

Gly Leu Asp Ile Leu Lys Asn Gln Thr Val Arg Gly Gly Thr Val Glu
     50                  55                  60

Met Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
 65                  70                  75                  80

Lys Glu Lys Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr
                 85                  90                  95

Glu Leu Arg Thr Arg Gln Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly
            100                 105                 110

Val Met Ser Thr Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val
        115                 120                 125

Pro Lys Val Ser Ala Ser His Leu Glu Glu Gly
    130                 135
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<210> 23
<211> 15
<212> PRT
<213> Clostridium tetani

<400> 23

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Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr Glu Leu
  1               5                  10                  15
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<210> 24
<211> 21
<212> PRT
<213> Clostridium tetani

<400> 24

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Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys Val Ser
  1               5                  10                  15

Ala Ser His Leu Glu
            20
```

CLAIMS

1. A method for *in vivo* down-regulation of interleukin 5 (IL-5) activity in an animal, including a human being, the method comprising effecting presentation to the animal's immune system of an immunogenically effective amount of

- at least one IL-5 polypeptide or subsequence thereof which has been formulated so that immunization of the animal with the IL-5 polypeptide or subsequence thereof induces production of antibodies against the IL-5 polypeptide, and/or
- at least one IL-5 analogue wherein is introduced at least one modification in the IL-5 amino acid sequence which has as a result that immunization of the animal with the analogue induces production of antibodies against the IL-5 polypeptide.

2. The method according to claim 1, wherein is presented an IL-5 analogue with at least one modification of the IL-5 amino acid sequence.

3. The method according to claim 2, wherein the modification has as a result that a substantial fraction of IL-5 B-cell epitopes are preserved and that

- at least one foreign T helper lymphocyte epitope (T_H epitope) is introduced, and/or
- at least one first moiety is introduced which effects targeting of the modified molecule to an antigen presenting cell (APC) or a B-lymphocyte, and/or
- at least one second moiety is introduced which stimulates the immune system, and/or
- at least one third moiety is introduced which optimizes presentation of the modified IL-5 polypeptide to the immune system.

4. The method according to claim 3, wherein the modification includes introduction as side groups, by covalent or non-

covalent binding to suitable chemical groups in IL-5 or a subsequence thereof, of the foreign T_H epitope and/or of the first and/or of the second and/or of the third moiety.

5. The method according to claim 3 or 4, wherein the modification includes amino acid substitution and/or deletion and/or insertion and/or addition.

6. The method according to claim 5, wherein the modification results in the provision of a fusion polypeptide.

7. The method according to claim 5 or 6, wherein introduction of the amino acid substitution and/or deletion and/or insertion and/or addition results in a substantial preservation of the overall tertiary structure of IL-5.

8. The method according to any one of claims 2-7, wherein the modification includes duplication of at least one IL-5 B-cell epitope and/or introduction of a hapten.

9. The method according to any one of claims 3-8, wherein the foreign T-cell epitope is immunodominant in the animal.

10. The method according to any one of claims 3-9, wherein the foreign T-cell epitope is promiscuous.

11. The method according to claim 10, wherein the at least one foreign T-cell epitope is selected from a natural promiscuous T-cell epitope and an artificial MHC-II binding peptide sequence.

12. The method according to claim 11, wherein the natural T-cell epitope is selected from a Tetanus toxoid epitope such as P2 or P30, a diphtheria toxoid epitope, an influenza virus hemaglutinin epitope, and a *P. falciparum* CS epitope.

13. The method according to any one of claims 3-12, wherein the first moiety is a substantially specific binding partner for a B-lymphocyte specific surface antigen or for an APC specific surface antigen.

14. The method according to claim 13, wherein the first moiety is a carbohydrate for which there is a receptor on the B-lymphocyte or the APC, such as mannan or mannose or wherein the first moiety is a hapten.

15. The method according to any one of claims 3-14, wherein the second moiety is selected from a cytokine, a hormone, and a heat-shock protein.

16. The method according to claim 6, wherein the cytokine is selected from, or is an effective part of, interferon γ (IFN-γ), Flt3L, interleukin 1 (IL-1), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 6 (IL-6), interleukin 12 (IL-12), interleukin 13 (IL-13), interleukin 15 (IL-15), and granulocyte-macrophage colony stimulating factor (GM-CSF).

17. The method according to claim 15, wherein the heat-shock protein is selected from the group consisting of HSP70, HSP90, HSC70, GRP94, and calreticulin (CRT), or an effective part thereof.

18. The method according to any one of claims 3-17, wherein the third moiety is of lipid nature, such as a palmitoyl group, a myristyl group, a farnesyl group, a geranyl-geranyl group, a GPI-anchor, and an N-acyl diglyceride group.

19. The method according to any one of the preceding claims, wherein the IL-5 polypeptide has been modified in at least one of loops 1-3 or in the amino acid residues C-terminal to helix D, said loops and said helix D corresponding to those shown in Fig. 3 for human and murine IL-5.

20. The method according to claim 19, wherein the IL-5 polypeptide is a human IL-5 polypeptide.

21. The method according to claim 20, wherein the human IL-5 polypeptide has been modified by substituting at least one amino acid sequence in SEQ ID NO: 1 with at least one amino acid sequence of equal or different length and containing a foreign T_H epitope, wherein substituted amino acid residues are selected from the group consisting of residues 87-90, residues 33-43, residues 59-64, residues 86-91, and residues 110-113.

22. The method according to any one of the preceding claims, wherein presentation to the immune system is effected by having at least two copies of the IL-5 polypeptide, the subsequence thereof or the modified IL-5 polypeptide covalently of non-covalently linked to a carrier molecule capable of effecting presentation of multiple copies of antigenic determinants.

23. The method according to any the preceding claims, wherein the IL-5 polypeptide, the subsequence thereof, or the modified IL-5 polypeptide has been formulated with an adjuvant which facilitates breaking of autotolerance to autoantigens.

24. The method according to claim 23, wherein the adjuvant is selected from the group consisting of an immune targeting adjuvant; an immune modulating adjuvant such as a toxin, a cytokine and a mycobacterial derivative; an oil formulation; a polymer; a micelle forming adjuvant; a saponin; an immunostimulating complex matrix (an ISCOM matrix); a particle; DDA; aluminium adjuvants; DNA adjuvants; γ-inulin; and an encapsulating adjuvant.

25. The method according to any one of the preceding claims, wherein an effective amount of the IL-5 polypeptide or the IL-5 analogue is administered to the animal via a route selected from the parenteral route such as the intradermal, the subdermal, the

intracutaneous, the subcutaneous, and the intramuscular routes; the peritoneal route; the oral route; the buccal route; the sublinqual route; the epidural route; the spinal route; the anal route; and the intracranial route.

26. The method according to claim 25, wherein the effective amount is between 0.5 µg and 2,000 µg of the IL-5 polypeptide, the subsequence thereof or the analogue thereof.

27. The method according to claim 25 or 26, which includes at least one administration of the IL-5 polypeptide or analogue per year, such as at least 2, at least 3, at least 4, at least 6, and at least 12 administrations per year.

28. The method according to any one of claims 25-27, wherein the IL-5 polypeptide or analogue is contained in a virtual lymph node (VLN) device.

29. The method according to any one of claims 1-24, wherein presentation of modified IL-5 to the immune system is effected by introducing nucleic acid(s) encoding the modified IL-5 into the animal's cells and thereby obtaining *in vivo* expression by the cells of the nucleic acid(s) introduced.

30. The method according to claim 29, wherein the nucleic acid(s) introduced is/are selected from naked DNA, DNA formulated with charged or uncharged lipids, DNA formulated in liposomes, DNA included in a viral vector, DNA formulated with a transfection-facilitating protein or polypeptide, DNA formulated with a targeting protein or polypeptide, DNA formulated with Calcium precipitating agents, DNA coupled to an inert carrier molecule, DNA encapsulated in chitin or chitosan, and DNA formulated with an adjuvant.

31. The method according to claim 29 or 30, wherein the nucleic acids are administered intraarterially, intraveneously, or by the routes defined in claim 25.

32. The method according to claim 30 or 31, wherein the nucleic acid(s) is/are contained in a VLN device.

33. The method according to any one of claims 30-32, which includes at least one administration of the nucleic acids per year, such as at least 2, at least 3, at least 4, at least 6, and at least 12 administrations per year

34. A method for treating and/or preventing and/or ameliorating asthma or other chronic allergic conditions characterized by eosinophilia, the method comprising down-regulating IL-5 activity according to the method of any one of claims 1-32 to such an extent that the number of eosinophil cells, either systemically or locally at the disease focus, is significantly reduced, such as a reduction of at least 20%.

35. An IL-5 analogue which is derived from an animal IL-5 polypeptide wherein is introduced a modification which has as a result that immunization of the animal with the analogue induces production of antibodies against the IL-5 polypeptide.

36. An IL-5 analogue according to claim 35, wherein the modification is as defined in any one of claims 1-22.

37. An immunogenic composition comprising an immunogenically effective amount of an IL-5 polypeptide autologous in an animal, said IL-5 polypeptide being formulated together with an immunologically acceptable adjuvant so as to break the animal's autotolerance towards the IL-5 polypeptide, the composition further comprising a pharmaceutically and immunologically acceptable carrier and/or vehicle.

38. An immunogenic composition comprising an immunogenically effective amount of an IL-5 analogue according to claim 35 or 36, the composition further comprising a pharmaceutically and immunologically acceptable carrier and/or vehicle and optionally an adjuvant.

39. An immunogenic composition according to Claim 37 or 38, wherein the adjuvant is selected from the group consisting of the adjuvants of claim 24.

40. A nucleic acid fragment which encodes an IL-5 analogue according to claim 35 or 36.

41. A vector carrying the nucleic acid fragment according to claim 40.

42. The vector according to claim 41 which is capable of autonomous replication.

43. The vector according to claim 41 or 42 which is selected from the group consisting of a plasmid, a phage, a cosmid, a mini-chromosome, and a virus.

44. The vector according to any one of claims 41-43, comprising, in the 5'→3' direction and in operable linkage, a promoter for driving expression of the nucleic acid fragment according to claim 40, optionally a nucleic acid sequence encoding a leader peptide enabling secretion of or integration into the membrane of the polypeptide fragment, the nucleic acid fragment according to claim 40, and optionally a terminator.

45. The vector according to any one of claims 41-44 which, when introduced into a host cell, is integrated in the host cell genome.

46. The vector according to any one of claims 41-44 which, when introduced into a host cell, is not capable of being integrated in the host cell genome.

47. The vector according to any one of claims 41-46, wherein the promoter drives expression in a eukaryotic cell.

48. The vector according to any one of claims 41-46, wherein the promoter drives expression in a prokaryotic cell.

49. A transformed cell carrying the vector of any one of claims 41-48.

50. The transformed cell according to claim 49 which is capable of replicating the nucleic acid fragment according to claim 40.

51. The transformed cell according to claim 50, which is a microorganism selected from a bacterium, a yeast, a protozoan, or a cell derived from a multicellular organism selected from a fungus, an insect cell such as an S_2 or an SF cell, a plant cell, and a mammalian cell.

52. The transformed cell according to claim 51 which is a bacterium of the genus *Escherichia*, *Bacillus*, *Salmonella*, or *Mycobacterium*.

53. The transformed cell according to claim 52, which is an *E. coli* cell.

54. The transformed cell according to claim 53, which is a non-pathogenic *Mycobacterium* cell such as *M. bovis* BCG.

55. The transformed cell according to any one of claims 49-54, which expresses the nucleic acid fragment according to claim 40.

56. The transformed cell according to claim 55, which secretes or carries on its surface, the IL-5 analogue according to claim 35 or 36.

57. The method according to any one of claims 1-22, wherein presentation to the immune system is effected by administering a non-pathogenic microorganism or virus which is carrying a nucleic acid fragment which encodes and expresses the IL-5 polypeptide or analogue.

58. The method according to claim 57, wherein the virus is a non-virulent pox virus such as a vaccinia virus.

59. The method according to claim 58, wherein the microorganism is a bacterium.

60. The method according to claim 58, wherein the bacterium is as defined in any one of claims 52-54.

61. The method according to any one of claims 57-60, wherein the non-pathogenic microorganism or virus is administered one single time to the animal.

62. A composition for inducing production of antibodies against IL-5, the composition comprising

- a nucleic acid fragment according to claim 40 or a vector according to any one of claims 41-48, and
- a pharmaceutically and immunologically acceptable carrier and/or vehicle and/or adjuvant.

63. The composition according to claim 62, wherein the nucleic acid fragment is formulated according to claim 30 or 32.

64. A stable cell line which carries the vector according to any one of claims 41-48 and which expresses the nucleic acid fragment according to claim 40, and which optionally secretes or

carries the IL-5 analogue according to claim 35 or 36 on its surface.

65. A method for the preparation of the cell according to any one of claims 49-56, the method comprising transforming a host cell with the nucleic acid fragment according to claim 40 or with the vector according to any one of claims 41-48.

66. A method for the identification of a modified IL-5 polypeptide which is capable of inducing antibodies against unmodified IL-5 in an animal species where the unmodified IL-5 polypeptide is a self-protein, the method comprising

- preparing, by means of peptide synthesis or genetic engineering techniques, a set of mutually distinct modified IL-5 polypeptides wherein amino acids have been added to, inserted in, deleted from, or substituted into the amino acid sequence of an IL-5 polypeptide of the animal species thereby giving rise to amino acid sequences in the set which comprise T-cell epitopes which are foreign to the animal species,
- testing members of the set for their ability to induce production of antibodies by the animal species against the unmodified IL-5, and
- isolating the member(s) of the set which significantly induces antibody production against unmodified IL-5 in the animal species.

67. A method for the preparation of an immunogenic composition comprising at least one modified IL-5 polypeptide which is capable of inducing antibodies against unmodified IL-5 in an animal species where the unmodified IL-5 polypeptide is a self-protein, the method comprising

- preparing, by means of peptide synthesis or genetic engineering techniques, a set of mutually distinct modified

IL-5 polypeptides wherein amino acids have been added to, inserted in, deleted from, or substituted into the amino acid sequence of an IL-5 polypeptide of the animal species thereby giving rise to amino acid sequences in the set comprising T-cell epitopes which are foreign to the animal,

- testing members of the set for their ability to induce production of antibodies by the animal species against the unmodified IL-5, and
- and admixing the member(s) of the set which significantly induces production of antibodies in the animal species which are reactive with IL-5 with a pharmaceutically and immunologically acceptable carrier and/or vehicle, optionally in combination with at least one pharmaceutically and immunologically acceptable adjuvant.

68. The method according to claim 66 or 67, wherein preparation of the members of the set comprises preparation of mutually distinct nucleic acid sequences, each sequence being a nucleic acid sequence according to claim 40, insertion of the nucleic acid sequences into appropriate expression vectors, transformation of suitable host cells with the vectors, and expression of the nucleic acid sequences, optionally followed by isolation of the expression products.

69. The method according to claim 68, wherein the preparation of the nucleic acid sequences and/or the vectors is achieved by the aid of a molecular amplification technique such as PCR

70. The method according to claim 68, wherein the preparation of the nucleic sequences and/or the vectors is achieved by the aid of nucleic acid synthesis.

71. Use of IL-5 or a subsequence thereof for the preparation of an immunogenic composition comprising an adjuvant for down-regulating IL-5 activity in an animal.

72. Use of IL-5 or a subsequence thereof for the preparation of an immunogenic composition comprising an adjuvant for the treatment, prophylaxis or amelioration of asthma or other chronic allergic conditions.

73. Use of an IL-5 analogue for the preparation of an immunogenic composition optionally comprising an adjuvant for down-regulating IL-5 activity in an animal.

74. Use of an IL-5 analogue for the preparation of an immunogenic composition optionally comprising an adjuvant for the treatment, prophylaxis or amelioration of asthma or other chronic allergic conditions.

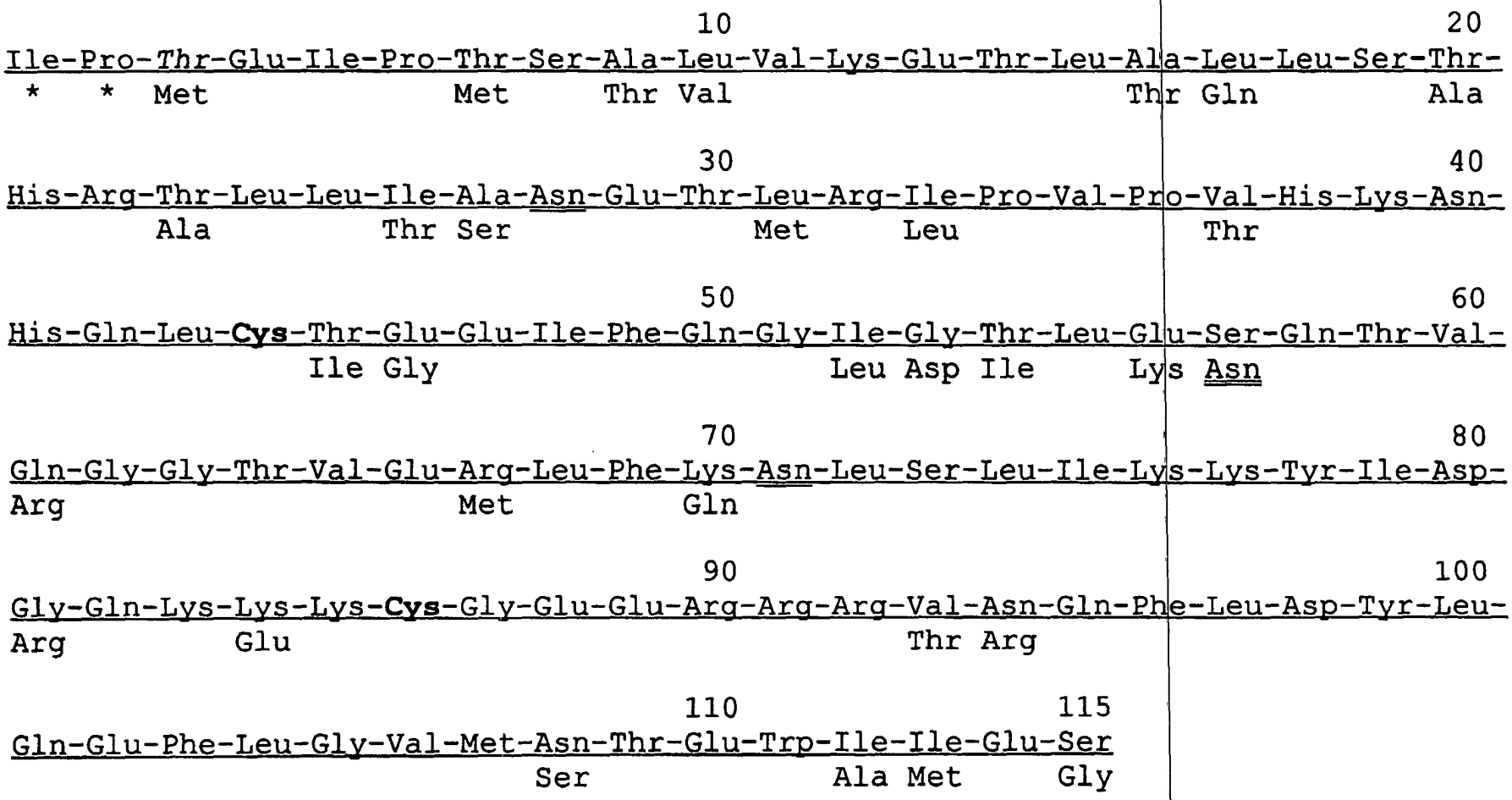

Fig. 1

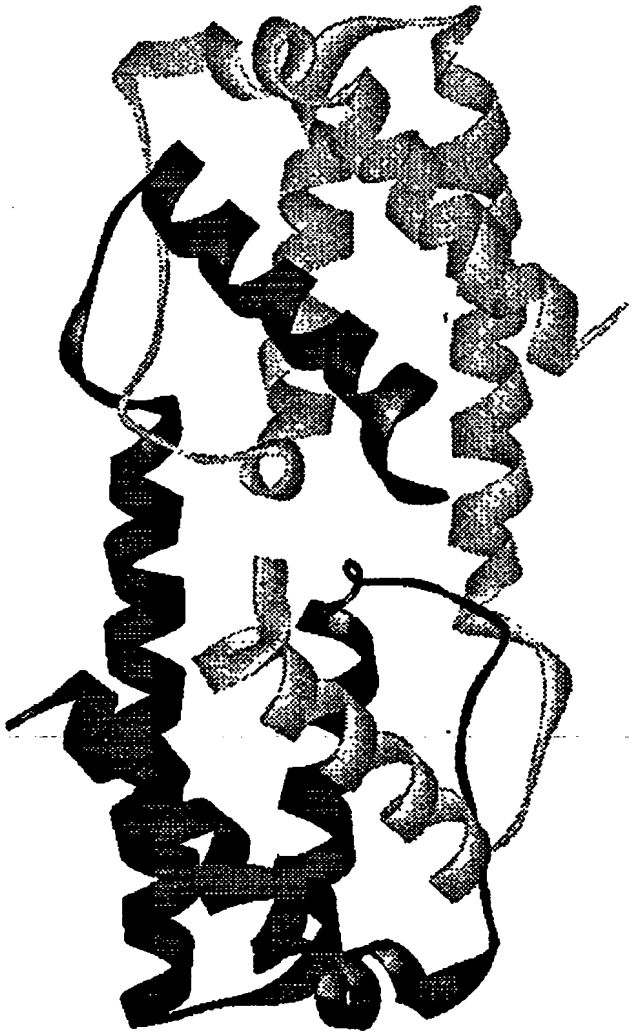

Fig. 2A

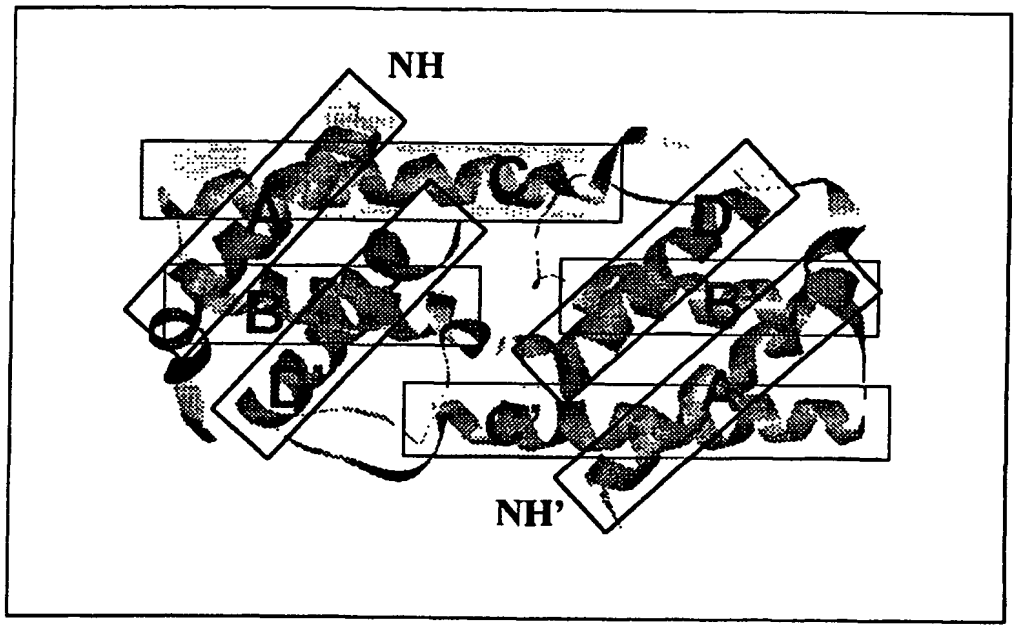

Fig. 2B

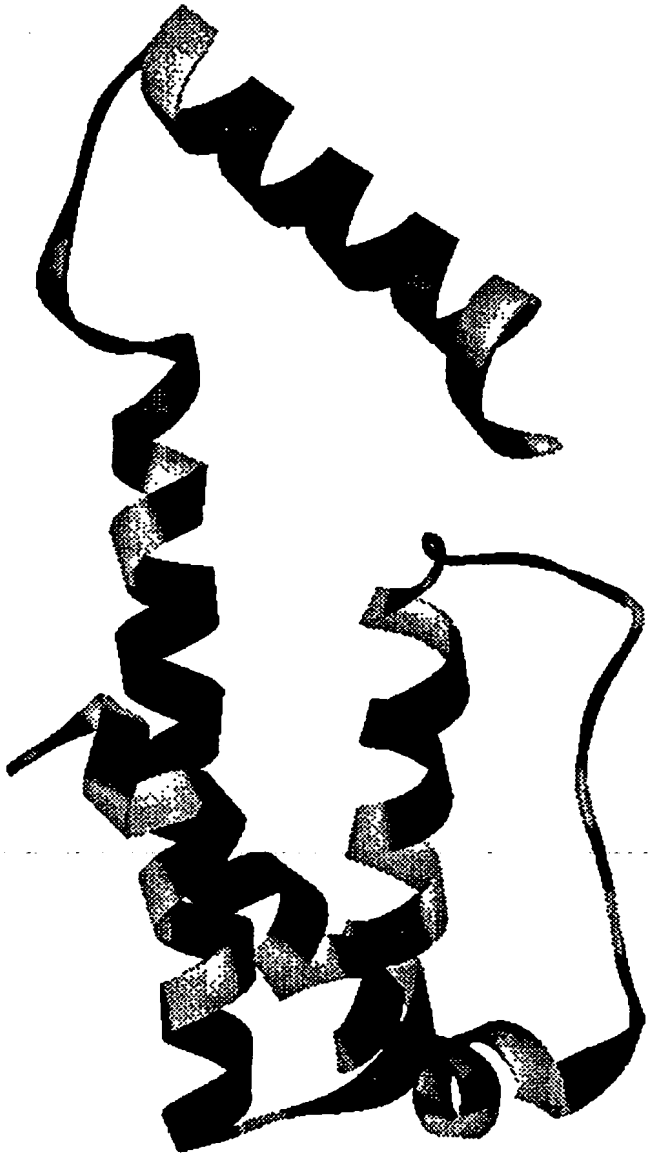

Fig. 2C

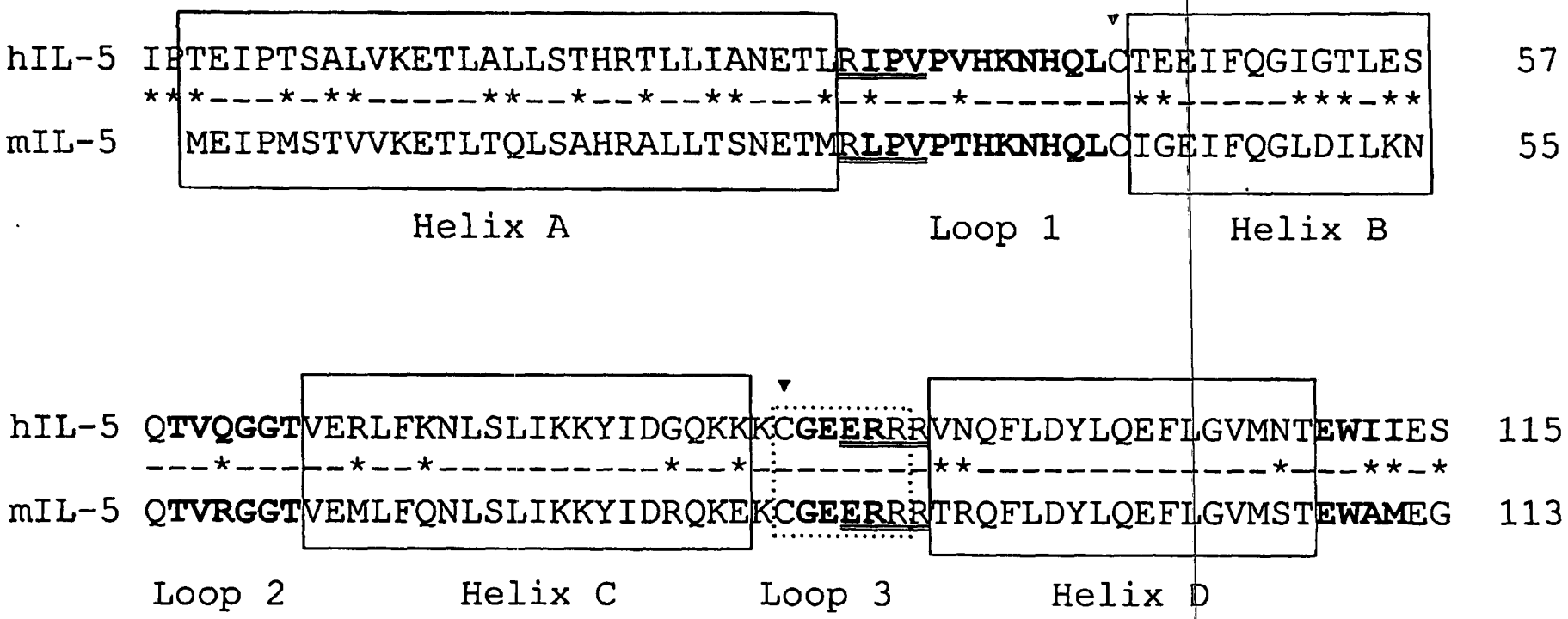

Fig. 3